

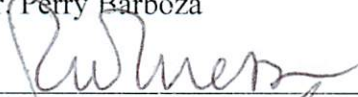
CAUSES AND CONSEQUENCES OF GEOPHAGY IN SNOWSHOE HARES  
(LEPUS AMERICANUS), AN IMPORTANT GENERALIST HERBIVORE  
OF THE BOREAL FOREST


By

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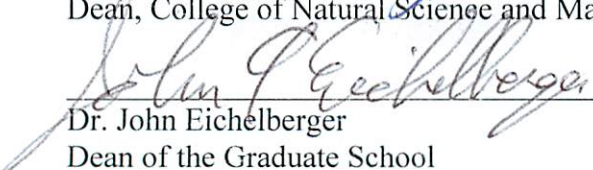
  
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CAUSES AND CONSEQUENCES OF GEOPHAGY IN SNOWSHOE HARES  
(*LEPUS AMERICANUS*), AN IMPORTANT GENERALIST HERBIVORE  
OF THE BOREAL FOREST

A  
THESIS

Presented to the Faculty  
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By

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## Abstract

Geophagy, the consumption of mineral soil, is believed to have several benefits for herbivores. Soils high in clay are often implicated in the detoxification of plant secondary metabolites. High mineral concentrations in soils may also provide nutrients that are poorly available from plants. Local observers report that snowshoe hares (*Lepus americanus*) use a lick in the foothills of the Brooks Range, Alaska. Using soil from this lick and other mineral supplements, I conducted a series of feeding trials on captive snowshoe hares fed felt-leaf willow (*Salix alaxensis*) or a formulated ration to determine whether geophagy resulted in a physiological benefit and, if so, which soil constituents are therapeutic. When fed willow leaves, hares ate more and lost less weight when they had access to soil. Access to soil increased sodium intake and dietary ratios of sodium to potassium in hares fed willow. Soil consumption resulted in higher calcium to phosphorous ratios for both diets. Across diets, higher sodium to potassium and lower calcium to phosphorus ratios corresponded to reduced weight loss. Access to pure calcium carbonate resulted in reduced weight loss in hares fed winter dormant willow twigs, suggesting that carbonates may also be an important component of this lick.



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## **Chapter 1 Introduction**

Geophagy, the consumption of mineral soil, has been reported in a wide range of species, including birds, ungulates and primates, and is particularly prevalent among generalist herbivores (Johns 1990). Geophagic behaviors among vertebrates have been attributed primarily to the acquisition of mineral nutrients and detoxification of plant secondary metabolites (PSMs; Krishnamani and Mahaney 2000; Slamova et al. 2011; Young et al. 2011).

Soil consumption can augment mineral intakes when forage plants provide insufficient supplies of minerals. In particular, sodium (Na) deficit is often reported as a cause of geophagy (e.g. Ayotte et al. 2006; Young et al. 2011; Dudley et al. 2012). Sodium is essential for many physiological processes, including maintaining acid-base balance, muscle contraction, membrane function and nerve impulse transmission. Requirements for Na also increase during growth and reproduction (Barboza et al. 2009). Because Na does not accumulate to high concentrations in most terrestrial plants, herbivores may seek Na from licks or other sources, especially during times of high physiological demand (Hui 2004). Licks have been shown to serve as concentrated sources of Na, particularly in non-coastal areas where aerosol deposition of Na from oceanic sources is precluded (Dudley et al. 2012).

Mineral licks often contain high concentrations of calcium (Ca). This element is often cited as the target element for many species of geophagic animals (Jones and Hanson 1985; Holl and Bleich 1987; Tracy and McNaughton 1995; Wilson 2003;

Abrahams 2005; Ayotte et al. 2006; Young et al. 2011). Calcium is involved in muscle contraction, nerve impulse transmission and metabolism, and is an essential element in the skeletal matrix, milk, antlers and egg shells (Barboza et al. 2009). Calcium is commonly added to livestock diets as a mineral supplement, and in the form of  $\text{CaCO}_3$  it has been shown to improve digestibility of dry matter and starch in ruminants (Varner and Woods 1972; Cullison 1975; James and Wohlt 1985) and increase food intake and weight gain in pigs (Patience and Wolynetz 1990). However, Ca or other minerals found in lick soil may also have detrimental effects resulting from mineral interactions and imbalances (Abrahams 2005).

In addition to ameliorating elemental deficiencies, consumption of soils may have a role in counteracting the negative effects of toxins such as phenolics and other PSMs. In particular, soils high in clay are thought to adsorb toxins, facilitating elimination through feces and preventing toxic effects. This may be particularly relevant for generalist herbivores that routinely cope with a range of PSMs.

Tannins, a class of polyphenolic compounds, are particularly common in woody plants and are often found in high concentrations in browse species. Tannins interact with proteins to form insoluble complexes, which reduce protein absorption, and may inhibit digestive enzymes (Bernays et al. 1989). Tannins are known to cause erosion of the epithelial cells of the small intestine, which results in the loss of Na, K (potassium) and other minerals (Freeland et al. 1985). Tannins have also been shown to reduce absorption of Ca (Chang et al. 1994; Al-Mamary et al. 2001; Hassan et al. 2003).

Snowshoe hares (*Lepus americanus*) are generalist herbivores that consume a variety of forages. During the summer months, hares consume herbaceous forages, including forbs, grasses and the leaves of deciduous shrubs. During fall and winter they browse twigs, buds and bark from woody species (Wolff 1978). Winter browse has lower nitrogen content compared to summer forages (Kubota et al. 1970), and is known to contain a variety of PSMs, which can limit intake and may increase nutrient requirements.

Local observations suggest that snowshoe hares in areas near Wiseman, Alaska (N 67.41, W 150.11) consume mineral soil at specific sites. Moreover, observers report that snowshoe hare populations in areas with a known lick appear to reach higher densities during the population high compared to areas where there is no known licks, a possibility noted by Klaus et al. (1998). These observations provided the motivation for the present study to examine the nutritional basis of geophagy. Chapter 2 examines causes and consequences of geophagy in snowshoe hares during summer. Chapter 3 investigates the role of Ca and carbonate as secondary benefits of lick use during winter.



## References

- Abrahams PW (2005) Geophagy and the involuntary ingestion of soil. In: Selinus O, Alloway BJ (eds) *Essentials of medical geology*. Elsevier Science, Amsterdam
- Al-Mamary M, Al-Habori M, Al-Aghbari A, Al-Obeidi A (2001) In vivo effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption. *Nutrition Research* 21:1393-1401
- Ayotte JB, Parker KL, Arocena JM, Gillingham MP (2006) Chemical composition of lick soils: Functions of soil ingestion by four ungulate species. *Journal of Mammalogy* 87:878-888
- Barboza PS, Parker KL, Hume ID (2009) *Integrative wildlife nutrition*. Springer-Verlag, Berlin ; New York
- Bernays EA, Driver GC, Bilgener M (1989) Herbivores and plant tannins. *Advances in Ecological Research* 19:263-302
- Chang MCJ, Bailey JW, Collins JL (1994) Dietary tannins from cowpeas and tea transiently alter apparent calcium absorption but not absorption and utilization of protein in rats. *Journal of Nutrition* 124:283-288
- Cullison A (1975) *Feed and Feeding*. Prentice-Hall, Reston, VA
- Dudley R, Kaspari M, Yanoviak SP (2012) Lust for Salt in the Western Amazon. *Biotropica* 44:6-9
- Freeland WJ, Calcott PH, Geiss DP (1985) Allelochemicals, minerals and herbivore population size. *Biochemical Systematics and Ecology* 13:195-206
- Hassan IAG, Elzubeir EA, El Tinay AH (2003) Growth and apparent absorption of minerals in broiler chicks fed diets with low or high tannin contents. *Tropical Animal Health and Production* 35:189-196
- Holl SA, Bleich VC (1987) Mineral lick use by mountain sheep in the San Gabriel Mountains, California. *Journal of Wildlife Management* 51:383-385
- Hui CA (2004) Geophagy and potential contaminant exposure for terrestrial vertebrates. *Reviews of Environmental Contamination and Toxicology*, vol 183, pp 115-134
- James LG, Wohlt JE (1985) Effect of Supplementing Equivalent Cation Amounts from NaCl, MgO, NaHCO<sub>3</sub> and CaCO<sub>3</sub> on Nutrient Utilization and Acid-Base Status of Growing Dorset Lambs Fed High Concentrate Diets. *Journal of Animal Science* 60:307-315
- Johns T (1990) *With bitter herbs they shall eat it : chemical ecology and the origins of human diet and medicine*. University of Arizona Press, Tucson
- Jones RL, Hanson HC (1985) Mineral licks, geophagy, and biogeochemistry of North American ungulates. The Iowa State University Press, Ames, IA
- Klaus G, Klaus-Hugi C, Schmid B (1998) Geophagy by large mammals at natural licks in the rain forest of the Dzanga National Park, Central African Republic. *Journal of Tropical Ecology* 14:829-839
- Krishnamani R, Mahaney WC (2000) Geophagy among primates: adaptive significance and ecological consequences. *Animal Behaviour* 59:899-915
- Kubota J, Rieger S, Lazar VA (1970) Mineral Composition of Herbage Browsed by Moose in Alaska. *The Journal of Wildlife Management* 34:565-569

- Patience JF, Wolynetz MS (1990) Influence of dietary undetermined anion on acid-base status and performance in pigs. *Journal of Nutrition* 120:579-587
- Slamova R, Trckova M, Vondruskova H, Zraly Z, Pavlik I (2011) Clay minerals in animal nutrition. *Applied Clay Science* 51:395-398
- Tracy BF, McNaughton SJ (1995) Elemental analysis of mineral lick soils from the Serengeti National Park, the Konza Prairie and Yellowstone National Park. *Ecography* 18:91-94
- Varner LW, Woods W (1972) Effect of Calcium and Starch Additions upon Ration Digestibility by Steers. *Journal of Animal Science* 35:410-414
- Wilson MJ (2003) Clay mineralogical and related characteristics of geophagic materials. *Journal of Chemical Ecology* 29:1525-1547
- Wolff JO (1978) Food habits of snowshoe hares in interior Alaska. *The Journal of Wildlife Management* 42:148-153
- Young SL, Sherman PW, Lucks JB, Pelto GH (2011) Why On Earth?: Evaluating Hypotheses About The Physiological Functions Of Human Geophagy. *The Quarterly Review of Biology* 86:97-120



## Chapter 2 Effects of geophagy on food intake, body mass, and nutrient dynamics of snowshoe hares (*Lepus americanus*) fed fresh willow leaves<sup>1</sup>

### Abstract

Geophagy, the consumption of mineral soil, is thought to have several physiological benefits to animals, including mineral supplementation and adsorption of toxins. Geophagy has often been reported for primates, birds and ungulates, but rarely for small mammals. Using soil from a known lick in northern Alaska, I investigated the effect of geophagy on food intake and weight loss in captive snowshoe hares (*Lepus americanus*) fed a formulated ration or felt-leaf willow leaves (*Salix alaxensis*), a preferred forage species of snowshoe hares. Lick soil contained 4.8% clay, 12.5% carbonates, and had more available Na (72.7 mg kg<sup>-1</sup>) than willow leaves. Hares fed willow consumed more soil ( $15.2 \pm 2.0$  g kg<sup>-0.75</sup> d<sup>-1</sup>) than those fed a formulated ration ( $9.3 \pm 1.3$  g kg<sup>-0.75</sup> d<sup>-1</sup>). When fed willow leaves, hares offered soil lost 0.44% body weight compared to 4.39% for hares without soil. Hares offered soil had higher daily willow intake ( $45.8 \pm 2.0$  g kg<sup>-0.75</sup> d<sup>-1</sup>) compared to hares without soil ( $35.0 \pm 2.1$  g kg<sup>-0.75</sup> d<sup>-1</sup>). Access to soil resulted in higher digestible intakes of N, Ca, Mg, Na, and K, but not P. Weight loss was associated with decreasing Na:K and increasing Ca:P intakes. The availability of mineral licks may alter forage use and functional response of generalist herbivores in interior Alaska.

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<sup>1</sup> Worker, SB, K Kielland, PS Barboza. 2013. Effect of geophagy on food intake, body mass, and nutrient dynamics of snowshoe hares (*Lepus americanus*) fed fresh willow leaves. Formatted for submission to *Oecologia*.

## **Introduction**

Geophagy, the consumption of mineral soil, has been reported in a wide range of species, including birds, ungulates and primates, and is particularly prevalent among generalist herbivores (Johns 1990). Geophagic behaviors among vertebrates have been attributed primarily to the acquisition of mineral nutrients and detoxification of plant secondary metabolites (PSMs) and may also have a role in the control of diarrhea and in pH adjustment of the gut (Krishnamani and Mahaney 2000; Slamova et al. 2011; Young et al. 2011).

Soil consumption can augment mineral intakes when plants provide insufficient supplies of minerals. In particular, sodium (Na) deficit is often reported as a cause of geophagy (e.g. Ayotte et al. 2006; Young et al. 2011; Dudley et al. 2012). Sodium is essential for many physiological processes, including maintaining acid-base balance, muscle contraction, membrane function and nerve impulse transmission, and Na requirements increase during growth and reproduction (Barboza et al. 2009). It does not accumulate in high concentrations in most plants, so herbivores may seek Na from salt licks or other sources, particularly during times of high physiological demand (Hui 2004). Many licks have been shown to serve as concentrated sources of Na, as well as of calcium (Ca), magnesium (Mg), and potassium (K; Tracy and McNaughton 1995; Wilson 2003; Ayotte et al. 2006). Whereas soil ingestion is often beneficial, this behavior may also have detrimental effects resulting from mineral interactions and imbalances (Abrahams 2005).

Consumption of mineral soil may also have a role in counteracting the negative effects of toxins such as phenolics and other PSMs (Krishnamani and Mahaney 2000). In particular, soils high in clay are thought to adsorb toxins, facilitating elimination through feces and preventing toxic effects. This may be particularly relevant for generalist herbivores, such as rodents and lagomorphs, which routinely cope with a range of PSMs.

PSMs are produced by many plant species as a defensive mechanism against herbivory (Freeland and Janzen 1974; Glendinning 2007). The concentration of these chemicals in plants varies by species, season, plant age, plant developmental phase, and plant part, but nearly all woody species contain potentially toxic PSMs (Bryant and Kuropat 1980; Bryant et al. 1991). The ubiquity of PSMs in nature means that many herbivores are forced to consume them, at a physiological cost (Glendinning 2007). Furthermore, the process of browsing often increases the concentrations of PSMs in plants, so increased browsing intensity may decrease the forage quality for herbivores (Bryant et al. 1991).

Tannins, a class of polyphenolic compounds, are particularly common in plants and are often found in high concentrations in browse species. Tannins interact with proteins to form insoluble complexes, which may reduce dietary protein absorption, inhibit digestive enzymes, or cause erosion of the intestinal epithelium, resulting in loss of Na, K and other minerals (Bernays et al. 1989).

Herbivores have evolved an array of adaptations to counter the effects of tannins and other PSMs (McArthur et al. 1991). Many PSMs are inactivated by forming complexes with other gut constituents. These complexes are less reactive or less easily

absorbed and can be excreted in the feces. Ingested secondary compounds that do not form complexes and are absorbed must be biotransformed via enzyme-facilitated reactions. Biotransformation results in production of organic acids, which must be buffered and excreted and can result in urinary loss of Ca, Na or other minerals (Pehrson 1983; Foley et al. 1995).

The rate of detoxification of PSMs is limited by enzymatic rates of degradation and the energetic and nutritional costs of detoxification. Consumption of forage by an herbivore is therefore limited by the amount of a specific toxin, as well as the total load of toxic compounds ingested (Freeland and Janzen 1974; Forbey et al. 2011). If geophagy is an effective mechanism for binding toxins or mediating physiological effects of PSMs, geophagy might allow higher intakes of chemically defended browse, and directly or indirectly affect growth, survival, and reproduction, which has important ecological consequences.

Snowshoe hares (*Lepus americanus*) are generalist herbivores, which consume a variety of forage species. During the summer months, they typically feed on herbaceous vegetation and the leaves of deciduous shrubs. During fall and winter they largely browse the twigs, buds and bark of deciduous shrubs and trees, as well as some evergreen species (Wolff 1978). Winter browse has reduced nitrogen concentration compared to summer forages (Kubota et al. 1970), but contains high concentrations of secondary compounds, which can limit intake and may increase nutrient requirements. As a result, winter forage is a critical factor affecting survival and population dynamics of snowshoe hares (Bryant 1981).

Local observations suggest that snowshoe hares in areas near Wiseman, Alaska (N 67.41, W 150.11) consume mineral soil at specific sites. Observers report that snowshoe hare populations in areas with known licks appear to reach higher densities during the population high compared to areas where there is no known lick. These observations provided the motivation for the present study to examine the nutritional ecology of hares in the context of geophagy.

I relied on captive snowshoe hares to explore the relationship between geophagy and diet composition, and to investigate the physiological consequences of geophagy. Specifically, I examined intake rates, body mass and nutrient digestibility in the presence and absence of geophagy to test the hypothesis that this behavior confers physiological benefits. I hypothesized that hares with access to soil would have higher daily digestible intake of nutrients and gain more weight than those without access to soil. I predicted that these effects would be more pronounced in hares fed willow leaves containing PSMs compared to those fed a balanced ration without PSMs.

## **Methods**

### *Animals*

Ten snowshoe hares were captured between 08-Oct-2010 and 05-Nov-2010 in the boreal forest at the Bonanza Creek Long Term Ecological Research site (N 64.70, W 148.28), approximately 20 km southwest of Fairbanks, Alaska. This site is located in the boreal forest and provides hare habitat similar to that adjacent to the Wiseman lick. Hares were captured and transported in wire cage traps (model 106, Tomahawk Live



Trap, Tomahawk, WI). All animals were housed individually at the University of Alaska Fairbanks (UAF) Biological Reserve in 1 m x 2 m outdoor pens. The pens were covered and partially enclosed so they were protected from precipitation but subject to natural temperature and light conditions. Pens had wood shavings on the floor and each hare had access to a 46 x 60 x 33 cm metabolism cage and a 40 x 26 x 18 cm plastic hutch for cover. During all non-experimental periods, hares were maintained on a pelleted herbivore ration devoid of PSMs (Barboza and Parker 2006), and were provided small amounts of fresh willow (*Salix* sp.) and birch (*Betula neoalaskana*) browse daily. Hares had *ad libitum* access to food and water. All procedures were approved by the Animal Care and Use Committee under UAF protocol #175963-6.

### *Lick Soil*

Soil was collected from a lick known by locals to be used by hares and other herbivores. The lick is located on the southern flank of the Brooks Range, approximately 10 km north of Wiseman, Alaska (N 67.49, W 150.05). It is comprised of a bluff situated at the edge of a small river (Fig 2.1a) and is covered in a mineral precipitate (Fig 2.1b). Approximately 20 kg of loose soil was collected from the lower edge of the bluff for use in captive trials. The soil was sifted through a 2 mm mesh, mixed, sampled for chemical analysis and frozen until needed for the feeding trials.

Soil samples were thawed, air-dried and subsampled to determine clay content, pH, calcium carbonate equivalent ( $\text{CaCO}_3$ ), cation exchange capacity (CEC), available

mineral (Na, K, Ca, Mg) and total mineral content (Na, K, Ca, Mg, P, Fe, Mn, Zn, Cu, Co, Cr, Mo).

Clay content was determined by dispersing soil in sodium pyrophosphate solution and measuring density with a hydrometer at 40 seconds and 2 hours (Klute 1986). Water suspensions were used for pH determinations, following the Long Term Ecological Research standards (Robertson et al. 1999). Concentrations of  $\text{CaCO}_3$  were estimated by acid neutralization (USDA 1954). Cation exchange capacity and available minerals were determined by extraction in ammonium acetate adjusted to pH 7 (Page 1982). To approximate the acidic conditions of the gastric stomach, some authors advocate the use of acidic extracting solutions such as Mehlich 3 to estimate available minerals (e.g. Ayotte et al. 2006; Brightsmith et al. 2008). Mehlich 3 extraction has the same extraction efficiency as ammonium acetate for Na and K, but Mehlich 3 yields 1.10 times more Ca and Mg than ammonium acetate (Ziadi and Tran 2008), so I adjusted the latter values accordingly. To determine total mineral concentration, samples were digested in a mixture of sulfuric, nitric, hydrochloric and perchloric acids (Coltrane and Barboza 2010) and assayed by Direct Coupled Plasma spectrometry (Iris DCP, Thermo Elemental, Cheshire, UK). Total N was determined using an elemental analyzer (TruSpec CN, LECO, St. Joseph, MI).

### *Feeding trial*

Ten hares were housed individually in 46 x 60 x 33 cm metabolism cages from 18 June to 18 August 2011. Cages had 1 cm mesh floors and fine mesh subfloors to separate

feces and urine. Water was offered ad libitum in cage bottles. Hares were acclimated to the cages for 14 days, until feed intake and body weights were stable.

Treatments consisted of two diets and two soil regimes. Diets were either 100% formulated diet (D-ration, Alaska Pet and Garden, Anchorage, AK), a high-protein, high-energy diet formulated to be similar to willow leaves, or 100% willow leaves (*S. alaxensis*). Fresh, juvenile-form leaves were collected daily for feeding and sampled for chemical analysis. Both diets were offered ad libitum and were offered sequentially, separated by a transition period (Fig 2.2). Soil regimes were (+) Soil or (-) Soil. During each of two trials, half the hares had access to soil (Fig 2.2). Soil was offered in bricks prepared by adding gelatin (Knox brand, Kraft Foods, Tarrytown, NY) to soil at rate of 1% by weight, which resulted in a soil N concentration of 0.23 %. Each hare received one soil brick (~35-40 g) per day during treatment periods.

Refused food and soil were collected daily to calculate intake. Feces were collected daily. Hares were weighed to the nearest gram at the beginning, middle, and end of each treatment to calculate percent change in body mass (SP4001, Ohaus Corporation, Parsippany, NJ).

#### *Chemical analysis and calculations*

Fecal samples were dried in a forced air oven at 55°C. Willow samples were freeze-dried. Dried samples were ground in a Wiley Mill (#20 screen). Soil samples were pulverized in a ball mill. Representative fecal samples were prepared for each animal by combining a proportionate mass from each daily sample into a 25 g analytical

sample. Ash and total mineral concentration (Ca, Mg, Na, K, P, N) of food, soil bricks and feces was determined as described above. Apparent digestible mineral intakes were calculated as the difference of each component in the diet and the feces. Ingestion of abrasive materials such as soil increases endogenous N losses (Young and Hume 2005) so I estimated metabolic fecal nitrogen (MFN) by running a neutral detergent fiber digest (NDF) on fecal samples to remove soluble N (Van Soest 1994). I then subtracted N in NDF residue from total fecal N to estimate MFN (Barboza and Parker 2006). Total phenolic concentration of willow was determined by extraction in 70% acetone followed by reaction with Folin-Ciocalteu reagent, as described by Ainsworth and Gillespie (2007). Tannin concentration was assessed by measuring the protein binding capacity, using the radial diffusion method (Graça and Bärlocher 2005).

All statistical analyses were executed using R (version 2.15.2; R Core Team 2012). Repeated measures using mixed effects models (Pinheiro et al. 2012) were used to compare daily food and mineral intakes and body mass change among diet and soil treatments. Pairwise contrasts for diet and soil treatments were corrected for multiple comparisons with a Bonferroni adjustment (Hothorn et al. 2008). Square root transformations were applied to mineral intakes and mineral intake ratios to correct for unequal variance related to the mean, and arcsine of the square root transformations were applied to data expressed as proportions (Quinn and Keough 2002). Average values are reported as mean  $\pm$  SE. Statistical significance was determined at  $\alpha < 0.05$ .

## Results

Mineral soil obtained from the lick was relatively low in clay (4.8%), contained 12.5% CaCO<sub>3</sub> and had a pH of 8.8 (Table 2.1). Although soil was high in total minerals, especially Ca and Mg (Table 2.1), available minerals in soil were lower than in willow leaves, except for Na, which was higher in soil than leaves (Table 2.2, Table 2.3). When hares were fed willow leaves, daily soil intake was significantly higher ( $P=0.008$ ) in hares fed willow leaves ( $15.2 \pm 2.0 \text{ g kg}^{-0.75}$ ) than those fed the formulated diet ( $9.3 \pm 1.3 \text{ g kg}^{-0.75}$ ).

Compared to the formulated diet, willow leaves were lower in N, Na and P, but contained approximately twice as much Ca. Consequently, the ratio of Ca:P in willows was greater than that of the formulated diet, while the ratio of Na:K was much lower than that of the formulated diet. Unlike the formulated diet, willow leaves also contained significant amounts of phenolic compounds and tannins (Table 2.3).

Soil availability did not affect food intake<sup>2</sup> (Fig 2.3) or digestible dry matter (DM) intake (Table 2.4) for hares fed the formulated diet. For hares fed willow, access to soil resulted in a 30% increase in food intake (Fig 2.3) and a similar increase in digestible DM intake (Table 2.4). For both diets, DM digestibility decreased when soil was available, but this did not result in a change in organic matter digestibility (Table 2.5). Between diets, there was no difference in food intake for hares with access to soil (Fig 2.3), though hares had higher digestible DM intake when fed the formulated diet (Table 2.4).

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<sup>2</sup> “Food intake” is used to describe intake of willow leaves or the formulated diet, while “dry matter intake” refers to total dietary intake, including soil.

Mean pre-trial body mass was  $1317 \pm 15$  g. Hares maintained or gained weight during the trials, except for those on the willow diet with no soil, which lost 4.4% of their body mass over the six-day trial (Fig 2.4). Within willow diet, hares without access to soil lost significantly more mass than those offered soil. Among hares not offered soil, those fed willow lost more mass than those fed the formulated diet. There was no difference in change in body mass when hares were fed formulated diet with or without soil.

Nitrogen digestibility and digestible N intake were higher in hares fed the formulated diet compared to those fed willow leaves. Within diet, availability of soil had no effect of digestible N intake, but access to soil resulted in lower N digestibility for hares fed willow (Table 2.4, Table 2.5). Daily MFN which, ranged from 262 to 305 mg  $\text{kg}^{-1}$ , did not differ among treatments ( $P=0.14$ ). Within the willow diet, MFN comprised a significantly lower proportion of total fecal N in hares with access to soil ( $0.38 \pm 0.02$  g  $\text{g}^{-1}$ ) compared to hares without access to soil ( $0.45 \pm 0.03$  g  $\text{g}^{-1}$ ;  $P=0.03$ ). There was no difference in the MFN proportion between soil treatments ( $0.73 \pm 0.01$  g  $\text{g}^{-1}$ ;  $P=1$ ) for hares fed the formulated diet. Across diets, MFN proportion decreased marginally with increasing soil intake ( $P=0.06$ ).

PSMs were not significant predictors of body mass change. When hares were fed willow, Na was the only significantly influential dietary constituent ( $P=0.001$ ). Across diets, Na ( $P<0.001$ ), K ( $P=0.001$ ) and P ( $P=0.02$ ) intake had significant positive relationships to change in body mass.

Digestible Na intake was higher for hares fed the formulated diet. For hares fed willow, access to soil resulted in significantly higher digestible intakes of Na ( $P=0.01$ ) and K ( $P<0.001$ ; Table 2.4). Mineral digestibility varied primarily with diet and was not significantly affected by the presence of soil except for K, which was more digestible in hares without access to soil (Table 2.5). Na:K intake was low for hares fed willow, but access to soil resulted in doubling of the ratio. Soil had no effect on Na:K for hares fed the formulated diet (Table 2.6). There was a significant positive relationship between Na:K intake rates and body mass change across diets ( $P<0.001$ , Fig 2.5) and within willow diet ( $P=0.003$ ).

Digestible Ca and Mg intakes were higher for hares fed willow than those fed the formulated diet. Within willow diet, access to soil resulted in significantly higher digestible intake rates for Ca ( $P=0.002$ ) and Mg ( $P<0.001$ ). Hares were in negative P balance when fed willow (Table 2.4). Digestible intakes of P were positive on the formulated diet but reduced by the provision of soil ( $P=0.05$ ). Increasing soil intake resulted in higher dietary Ca:P ratios across diets ( $P=0.02$ ). Ca:P intake was different among all treatments ( $P<0.001$ ; Table 2.6) and was higher for hares on the willow diet. Across diets, change in body mass was negatively associated with Ca:P ( $P=0.03$ ; Fig 2.5).

## **Discussion**

### *Physiological effects*

Many lick soils associated with PSM detoxification contain high concentrations of clay, which confer a high adsorption capacity and potential for binding with tannins to

reduce the formation of tannin-protein complexes (Kreulen 1985; Johns and Duquette 1991), resulting in increased N digestibility. However, soil used in this experiment was several-fold lower in clay compared to many licks discussed in the scientific literature (e.g. Klaus et al. 1998; Gilardi et al. 1999; Wilson 2003; Ayotte et al. 2006; Brightsmith et al. 2008) and there is no evidence to suggest access to soil improved utilization of forage N (Table 2.4, 2.5). Furthermore tannin intake was not a significant driver of weight loss, supporting the conclusion that geophagy does not directly mediate tannins. However, clay may provide benefits not quantified in this experiment and may contribute to hares' drive to consume soil.

High roughage diets can have an abrasive effect on the GI tract, resulting in endogenous N loss. Soil ingestion can be expected to have a similar effect (Young and Hume 2005). High concentrations of dietary tannins can also result in endogenous N loss if unbound tannins bind with epithelial proteins (Freeland et al. 1985). The latter effect could be mediated through the formation of tannin-soil complexes, preventing epithelial erosion. My MFN data do not support either of these conclusions, indicating instead that soil and/or willow consumption was neither harmful nor beneficial with regard to preserving endogenous N. Increased fecal N observed in hares fed willow is of dietary origin is likely comprised of fiber-bound N in plant cell walls.

It is known that soils, particularly those containing clay, are capable of binding proteins (Shan 2011). Some researchers have suggested that soil particles may compete with tannins for the protein substrate (Kreulen 1985). If clay-protein complexes dissociate before they reach absorption sites, this mechanism could protect dietary protein



from binding with tannins and improve N utilization. However, if these complexes remain insoluble, it would result in a reduction of digestible N. The latter outcome is consistent with my observation of reduced N digestibility. Characterizing the potential for these complexes to form and dissociate is beyond the scope of this paper (Shan 2011), but these complexes may explain the reduction of N digestibility in hares fed soil. Hindrance of N utilization is a detriment to free-ranging herbivores but is apparently outweighed by the other benefits conferred by geophagy, including increased forage intake and mineral acquisition.

Terrestrial vegetation is generally low in Na and absorption can be impaired by excess K (Underwood and Suttle 2001; Barboza et al. 2009). PSMs, including tannins, are known to induce Na wasting in herbivores (Pehrson 1983; Freeland et al. 1985; Foley et al. 1995), exacerbating Na deficiencies. The estimated available Na concentration of lick soil was higher than that of willow leaves fed during the trial (Table 2.2, Table 2.3). Although soil Na concentration was lower than the dietary Na concentration considered adequate for domestic rabbits (0.2% DM; National Research Council 1977), provision of soil resulted in higher digestible Na intake (Table 2.4). Total Na intake in hares fed willow increased from  $1.3 \text{ mg kg}^{-0.75}$  without soil to  $4.2 \text{ mg kg}^{-0.75}$  with soil. However, the highest intakes of Na in hares fed willow were still lower than the general Na guidelines for wildlife of  $9.6 \text{ mg kg}^{-0.75}$  for an animal weighing 1300 g (Robbins 1993).

Across diets, there was a significant positive relationship between increasing Na:K intake and body mass (Fig 2.5a). Access to soil increased ingested Na:K (Table 2.6) for hares fed willow, though these ratios remained well below 0.33, the

recommended dietary Na:K ratio for domestic rabbits (National Research Council 1977). Pehrson (1983) reported that Na loss coincides with weight loss in hares. Likewise, dietary Na restriction can result in decreased apparent dry matter and crude protein digestibility (Chamorro et al. 2007). These findings are consistent with my observations. Given the importance of Na to physiological processes and the interactive constraint with PSMs, enhanced Na nutrition likely explains, at least in part, the benefits of soil consumption. It may also help explain the high daily soil intake rates. Although estimates of soil ingestion in free ranging mammals are scarce, soil generally comprises <10% of the diet of small herbivores (Beyer et al. 1994). This is well below my observations for hares eating willow, where soil comprised nearly 25% of total DM intake.

Soil used for my study was relatively high in Ca and Mg, similar to soil from many licks (Klaus et al. 1998; Wilson 2003; Abrahams 2005; Ayotte et al. 2006). For hares fed willow, soil ingestion resulted in digestible Ca and Mg intakes at least double that of hares not offered soil (Table 2.4). It's unclear to what degree these minerals are metabolized, however. Rabbits and hares are known to tolerate high serum levels of Ca, much of which is excreted in urine (National Research Council 1977; Goad et al. 1989) and urinary Ca has been shown to increase as dietary Ca increases (Clauss et al. 2011). Similarly, Mg is readily absorbed, with urine being the major route of excretion (National Research Council 1977; Pehrson 1983).

Hares fed willow were in negative P balance, regardless of soil treatment. Availability of P in plants is variable and can be negatively influenced by the presence of

PSMs and by high concentrations of Ca (Barboza et al. 2009). Increasing Ca:P ratios with soil availability corresponded to reduced P retention within both diets (Table 2.5, Table 2.6) and to increasing weight loss across all treatments (Fig 2.5b). Rabbits can tolerate dietary Ca:P ratios as high as 12:1 when both nutrients are present in sufficient quantities (National Research Council 1977). However, since hares eating willow were losing P, the high Ca:P ratios indicate a true imbalance, suggesting that soil ingestion may actually be detrimental to P nutrition in hares eating willow.

Apart from the imbalance between Ca and P, mineral toxicity did not appear to be an issue for hares engaging in geophagy. Intake rates of Cu, Co, Cr, Mn, Mo and Zn were well below the maximum tolerable level for livestock and laboratory animals (National Research Council 2005). Iron (Fe) intake from soil was higher than the maximum tolerable level for animals. However, these guidelines assume a highly digestible iron source (National Research Council 2005) and Fe availability of soil is expected to be very low. Furthermore, Fe absorption is low when Fe intake is high, and Ca appears to inhibit Fe absorption, suggesting that Fe toxicity is unlikely (National Research Council 2005).

This study shows a net benefit for hares engaging in geophagy, but this behavior can be both beneficial and detrimental (Hui 2004; Abrahams 2005). My data illustrates the trade-offs between enhanced Na acquisition, reduced N digestibility and accelerated P depletion resulting from excess Ca ingestion.

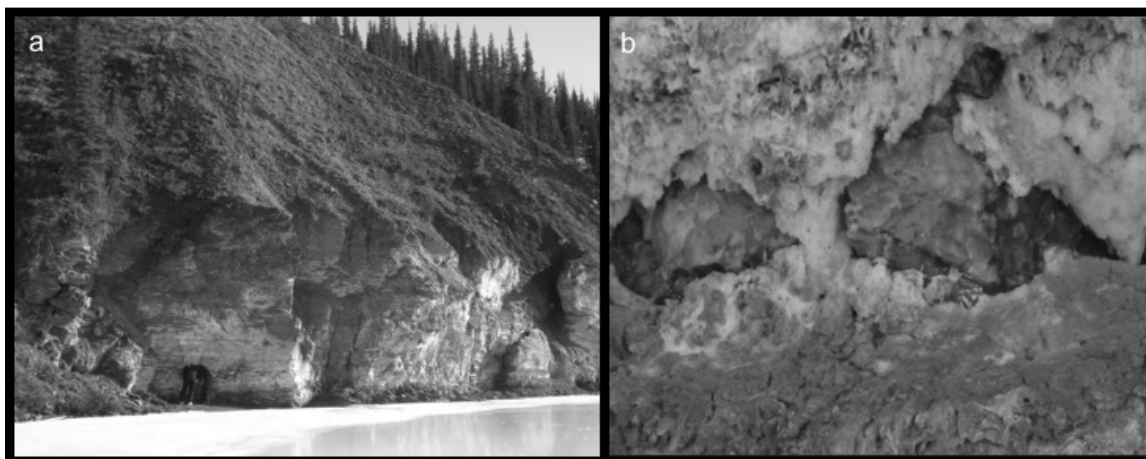
### *Ecological implications*

These trials suggest that soil ingestion provides a benefit to hares feeding on natural summer diets. Physiological demand is high for hares in summer. Energy and protein requirements increase for reproductive animals, as do requirements for mineral nutrients, including Na, K, Ca and P (National Research Council 1977; Barboza and Parker 2006). In winter, hares switch to woody browse, which is low in energy, high in PSMs, and may become scarce during high hare densities. This situation, coupled with increased thermoregulatory demands at low temperatures, results in a particular nutritional challenge for hares. If geophagy results in higher forage consumption rates or more efficient nutrient assimilation, this behavior likely represents an important adaptive response for reproduction and survival.

Changes in snowshoe hare reproduction and survival may have implications ecosystem-wide. Hares are considered a keystone species of the boreal forest and their abundance affects both vegetation communities and predator densities (Bryant et al. 1991; Rohner 1995; Stenseth et al. 1997; O'Donoghue et al. 1998; Krebs et al. 2001). Given that food resources play a role in regulating snowshoe hare population cycles (Bryant 1981; Krebs et al. 2001), and the potential for geophagy to alter use of food resources, licks may represent an important resource on the landscape. While the lick I studied is prominent, it is likely that many smaller, undetected licks exist in this area. It is unknown how prevalent these licks are, how they affect animal movement and spatial use of the food resources, and whether the benefits shown in my study are likely to be localized or widespread. Notwithstanding these uncertainties, the abundance and

distribution of licks may be an important factor controlling the local abundance and distribution of snowshoe hares in parts of interior Alaska.

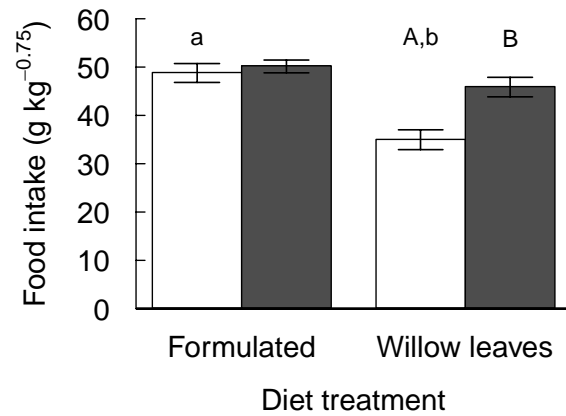
## Figures



**Fig 2.1** Bluff known by locals to be used as a lick by snowshoe hares and moose. a) Note two researchers at lower left for scale. b) The surface of the lick is covered in a soft white precipitate containing high Mg concentrations.

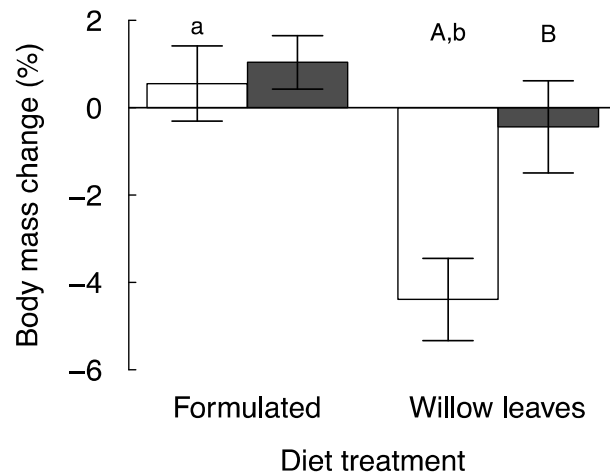
<b>Duration (days)</b>	<b>14</b>	<b>6</b>	<b>10</b>	<b>6</b>	<b>14</b>	<b>6</b>	<b>10</b>	<b>6</b>
<b>Group 1 n = 5</b>	Cage Acclimation	Formulated (+) Soil	Transition to Willow	Willow (+) Soil	Rest	Formulated (-) Soil	Transition to Willow	Willow (-) Soil
<b>Group 2 n = 5</b>		Formulated (-) Soil		Willow (-) Soil		Formulated (+) Soil		Willow (+) Soil

**Fig 2.2** Experimental design. Shaded areas indicate collection periods.

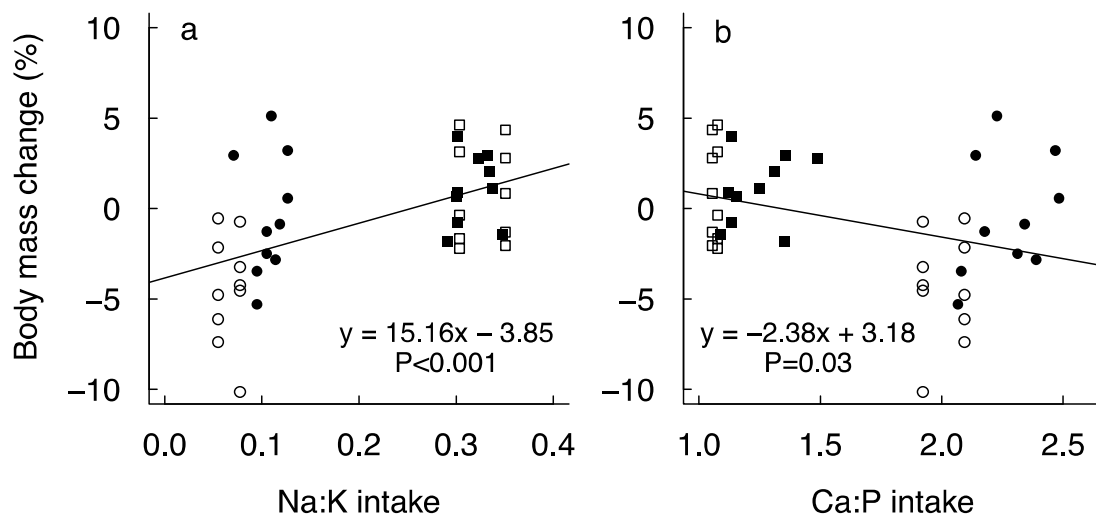


**Fig 2.3** Daily food intake (g dry mass kg<sup>-0.75</sup>) of snowshoe hares without access (open bars) or with access (filled bars) to lick soil (mean  $\pm$  SE; n=10). Different capital letters denote significant difference between soil treatments within diet ( $P \leq 0.001$ ). Different lower case letters denote significant difference between diet treatments within soil ( $P \leq 0.001$ ).





**Fig 2.4** Change in body mass of snowshoe hares without access (open bars) or with access (filled bars) to lick soil over a six-day experimental period (mean  $\pm$  SE;  $n=10$ ). Different capital letters denote significant difference between soil treatments within diet ( $P \leq 0.003$ ). Different lower case letters denote significant difference between diet treatments within soil ( $P \leq 0.001$ ).



**Fig 2.5** Regressions of change in body mass (%) against a) the square root of ingested Na:K and b) the square root of ingested Ca:P for hares fed willow (circles) or formulated diet (squares), with (filled symbols) and without (open symbols) access to soil (n=10).

## Tables

**Table 2.1** Soil properties from a lick used by herbivores near Wiseman, Alaska (n=5, except CaCO<sub>3</sub> n=3).

	Mean $\pm$ SE
pH	8.9 $\pm$ 0.0
CaCO <sub>3</sub> (%)	12.5 $\pm$ 0.4
Clay (%)	4.8 $\pm$ 0.2
CEC (meq.100 g <sup>-1</sup> )	1.4 $\pm$ 0.1
Na (mg kg <sup>-1</sup> )	313 $\pm$ 16
K (mg kg <sup>-1</sup> )	6492 $\pm$ 246
Ca (mg kg <sup>-1</sup> )	14701 $\pm$ 717
Mg (mg kg <sup>-1</sup> )	14537 $\pm$ 246
P (mg kg <sup>-1</sup> )	802 $\pm$ 12
Fe (mg kg <sup>-1</sup> )	47131 $\pm$ 428
Mn (mg kg <sup>-1</sup> )	2338 $\pm$ 31
Zn (mg kg <sup>-1</sup> )	118 $\pm$ 20
Cu (mg kg <sup>-1</sup> )	30.8 $\pm$ 1.2
Co (mg kg <sup>-1</sup> )	14.2 $\pm$ 4.7
Cr (mg kg <sup>-1</sup> )	84.2 $\pm$ 1.9
Mb (mg kg <sup>-1</sup> )	0 $\pm$ 0

**Table 2.2** Available minerals in lick soil (n=5).

	Mean $\pm$ SE
Na (mg g <sup>-1</sup> )	0.07 $\pm$ 0.001
K (mg g <sup>-1</sup> )	0.03 $\pm$ 0.002
Ca (mg g <sup>-1</sup> )	3.17 $\pm$ 0.03
Mg (mg g <sup>-1</sup> )	1.93 $\pm$ 0.02

**Table 2.3** Mineral and PSM constituents of experimental diets (mean  $\pm$  SE; n=3 for minerals, n=12 for PSMs). Different lower case letters denote significant difference between diets ( $P \leq 0.03$ ).

	Formulated diet	Willow leaves
N (mg g <sup>-1</sup> )	23.72 $\pm$ 0.25 <sup>a</sup>	21.75 $\pm$ 0.63 <sup>b</sup>
Na (mg g <sup>-1</sup> )	1.09 $\pm$ 0.12 <sup>a</sup>	0.05 $\pm$ 0.01 <sup>b</sup>
K (mg g <sup>-1</sup> )	10.18 $\pm$ 0.11	11.65 $\pm$ 0.81
Ca (mg g <sup>-1</sup> )	5.78 $\pm$ 0.11 <sup>a</sup>	10.51 $\pm$ 0.90 <sup>b</sup>
Mg (mg g <sup>-1</sup> )	1.77 $\pm$ 0.01	3.56 $\pm$ 0.58
P (mg g <sup>-1</sup> )	5.09 $\pm$ 0.18 <sup>a</sup>	2.59 $\pm$ 0.10 <sup>b</sup>
Na:K	0.10 $\pm$ 0.009 <sup>a</sup>	0.004 $\pm$ 0.001 <sup>b</sup>
Ca:P	1.14 $\pm$ 0.01 <sup>a</sup>	4.05 $\pm$ 0.20 <sup>b</sup>
Total Phenolics (mg g <sup>-1</sup> )	-	89.56 $\pm$ 1.14
Tannin (mg g <sup>-1</sup> )	-	61.05 $\pm$ 1.66

**Table 2.4** Digestible intakes of hares fed formulated diet or willow leaves, with and without access to lick soil (mean  $\pm$  SE; n=10). Different capital letters denote significant difference between soil treatments within diet ( $P \leq 0.01$ ). Different lower case letters denote significant difference between diet treatments within soil ( $P \leq 0.01$ ).

	Formulated diet		Willow leaves	
	No Soil	Soil	No Soil	Soil
Dry Matter ( $\text{g kg}^{-0.75} \text{ d}^{-1}$ )	32.8 $\pm$ 1.3 <sup>a</sup>	34.2 $\pm$ 1.1 <sup>a</sup>	21.1 $\pm$ 1.3 <sup>A,b</sup>	28.4 $\pm$ 1.2 <sup>B,b</sup>
N ( $\text{g kg}^{-0.75} \text{ d}^{-1}$ )	1.00 $\pm$ 0.04 <sup>a</sup>	1.05 $\pm$ 0.03 <sup>a</sup>	0.43 $\pm$ 0.03 <sup>b</sup>	0.50 $\pm$ 0.03 <sup>b</sup>
Ca ( $\text{mg kg}^{-0.75} \text{ d}^{-1}$ )	93.1 $\pm$ 16.9 <sup>a</sup>	166.7 $\pm$ 30.2 <sup>a</sup>	197.6 $\pm$ 41.6 <sup>A,b</sup>	410.7 $\pm$ 53 <sup>B,b</sup>
Mg ( $\text{mg kg}^{-0.75} \text{ d}^{-1}$ )	39.4 $\pm$ 4.7 <sup>A,a</sup>	107.8 $\pm$ 22.8 <sup>B,a</sup>	86.0 $\pm$ 13.7 <sup>A,b</sup>	236.9 $\pm$ 33.7 <sup>B,b</sup>
P ( $\text{mg kg}^{-0.75} \text{ d}^{-1}$ )	59.4 $\pm$ 9.8 <sup>a</sup>	25.6 $\pm$ 6.0 <sup>a</sup>	-20.9 $\pm$ 5.4 <sup>b</sup>	-35.6 $\pm$ 6.3 <sup>b</sup>
Na ( $\text{mg kg}^{-0.75} \text{ d}^{-1}$ )	47.1 $\pm$ 4.3 <sup>a</sup>	48.8 $\pm$ 3.7 <sup>a</sup>	1.3 $\pm$ 0.3 <sup>A,b</sup>	4.2 $\pm$ 0.5 <sup>B,b</sup>
K ( $\text{mg kg}^{-0.75} \text{ d}^{-1}$ )	429.4 $\pm$ 15.0	427.8 $\pm$ 11.3	368.7 $\pm$ 21.4 <sup>A</sup>	484.7 $\pm$ 24.3 <sup>B</sup>

**Table 2.5** Daily digestibilities in hares fed formulated diet or willow leaves, with and without access to lick soil (mean  $\pm$  SE; n=10). Different capital letters denote significant difference between soil treatments within diet ( $P \leq 0.001$ ). Different lower case letters denote significant difference between diet treatments within soil ( $P \leq 0.04$ ).

	Formulated diet		Willow leaves	
	No Soil	Soil	No Soil	Soil
Dry Matter	0.69 $\pm$ 0.01 <sup>A,a</sup>	0.58 $\pm$ 0.02 <sup>B,a</sup>	0.60 $\pm$ 0.01 <sup>A,b</sup>	0.47 $\pm$ 0.02 <sup>B,b</sup>
Organic Matter	0.70 $\pm$ 0.01 <sup>a</sup>	0.69 $\pm$ 0.01 <sup>a</sup>	0.60 $\pm$ 0.01 <sup>b</sup>	0.58 $\pm$ 0.01 <sup>b</sup>
N	0.87 $\pm$ 0.01 <sup>a</sup>	0.87 $\pm$ 0.01 <sup>a</sup>	0.60 $\pm$ 0.01 <sup>A,b</sup>	0.51 $\pm$ 0.02 <sup>B,b</sup>
Ca	0.31 $\pm$ 0.05 <sup>a</sup>	0.38 $\pm$ 0.04 <sup>a</sup>	0.48 $\pm$ 0.08 <sup>b</sup>	0.58 $\pm$ 0.04 <sup>b</sup>
Mg	0.45 $\pm$ 0.04 <sup>a</sup>	0.47 $\pm$ 0.04 <sup>a</sup>	0.65 $\pm$ 0.04 <sup>b</sup>	0.62 $\pm$ 0.03 <sup>b</sup>
P	0.23 $\pm$ 0.03 <sup>a</sup>	0.09 $\pm$ 0.02 <sup>a</sup>	-0.27 $\pm$ 0.08 <sup>b</sup>	-0.28 $\pm$ 0.05 <sup>b</sup>
Na	0.86 $\pm$ 0.03 <sup>a</sup>	0.84 $\pm$ 0.03 <sup>a</sup>	0.65 $\pm$ 0.07 <sup>b</sup>	0.58 $\pm$ 0.04 <sup>b</sup>
K	0.86 $\pm$ 0.01 <sup>A,a</sup>	0.77 $\pm$ 0.01 <sup>B</sup>	0.91 $\pm$ 0.01 <sup>A,b</sup>	0.77 $\pm$ 0.02 <sup>B</sup>

**Table 2.6** Mineral intake ratios for hares fed formulated diet or willow leaves, with and without access to lick soil (mean  $\pm$  SE; n=10). Different capital letters denote significant difference in comparisons of soil within diet treatments. Different lower case letters denote significant difference in comparisons of diet within soil treatments (P<0.001).

	Formulated diet		Willow leaves	
	No Soil	Soil	No Soil	Soil
Ca:P	1.14 $\pm$ 0.007 <sup>A,a</sup>	1.55 $\pm$ 0.11 <sup>B,a</sup>	4.04 $\pm$ 0.11 <sup>A,b</sup>	5.17 $\pm$ 0.22 <sup>B,b</sup>
Na:K	0.108 $\pm$ 0.005 <sup>a</sup>	0.101 $\pm$ 0.004 <sup>a</sup>	0.005 $\pm$ 0.001 <sup>A,b</sup>	0.012 $\pm$ 0.001 <sup>B,b</sup>



## References

- Abrahams PW (2005) Geophagy and the involuntary ingestion of soil. In: Selinus O, Alloway BJ (eds) Essentials of medical geology. Elsevier Science, Amsterdam
- Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protocols* 2:875-877
- Ayotte JB, Parker KL, Arocena JM, Gillingham MP (2006) Chemical composition of lick soils: Functions of soil ingestion by four ungulate species. *Journal of Mammalogy* 87:878-888
- Barboza PS, Parker KL (2006) Body protein stores and isotopic indicators of N balance in female reindeer (*Rangifer tarandus*) during winter. *Physiological and Biochemical Zoology* 79:628-644
- Barboza PS, Parker KL, Hume ID (2009) Integrative wildlife nutrition. Springer-Verlag, Berlin ; New York
- Bernays EA, Driver GC, Bilgener M (1989) Herbivores and plant tannins. *Advances in Ecological Research* 19:263-302
- Beyer WN, Connor EE, Gerould S (1994) Estimates of soil ingestion by wildlife. *Journal of Wildlife Management* 58:375-382
- Brightsmith DJ, Taylor J, Phillips TD (2008) The roles of soil characteristics and toxin adsorption in avian geophagy. *Biotropica* 40:766-774
- Bryant JP (1981) Phytochemical deterrence of snowshoe hare browsing by adventitious shoots of 4 Alaskan trees. *Science* 213:889-890
- Bryant JP, Kuropat PJ (1980) Selection of winter forage by sub-arctic browsing vertebrates - the role of plant chemistry. *Annual Review of Ecology and Systematics* 11:261-285
- Bryant JP, Provenza FD, Pastor J, Reichardt PB, Clausen TP, Dutoit JT (1991) Interactions between woody-plants and browsing mammals mediated by secondary metabolites. *Annual Review of Ecology and Systematics* 22:431-446
- Chamorro S, Gomez-Conde MS, Carabano R, de Blas JC (2007) Short communication. Low dietary sodium content affects the digestibility of nutrients and fattening performance in growing rabbits. *Spanish Journal of Agricultural Research* 5:470-473
- Clauss M et al. (2011) Influence of diet on calcium metabolism, tissue calcification and urinary sludge in rabbits (*Oryctolagus cuniculus*). *Journal of Animal Physiology and Animal Nutrition*:798-807
- Coltrane JA, Barboza PS (2010) Winter as a nutritional bottleneck for North American porcupines (*Erethizon dorsatum*).
- Dudley R, Kaspari M, Yanoviak SP (2012) Lust for Salt in the Western Amazon. *Biotropica* 44:6-9
- Foley WJ, McLean S, Cork SJ (1995) Consequences of biotransformation of plant secondary metabolites on acid-base metabolism in mammals - a final common pathway. *Journal of Chemical Ecology* 21:721-743

- Forbey JS, Pu XZ, Xu D, Kielland K, Bryant J (2011) Inhibition of snowshoe hare succinate dehydrogenase activity as a mechanism of deterrence for papyriferic acid in birch. *Journal of Chemical Ecology* 37:1285-1293
- Freeland WJ, Calcott PH, Geiss DP (1985) Allelochemicals, minerals and herbivore population size. *Biochemical Systematics and Ecology* 13:195-206
- Freeland WJ, Janzen DH (1974) Strategies in Herbivory by Mammals: The Role of Plant Secondary Compounds. *The American Naturalist* 108:269-289
- Gilardi JD, Duffey SS, Munn CA, Tell LA (1999) Biochemical functions of geophagy in parrots: Detoxification of dietary toxins and cytoprotective effects. *Journal of Chemical Ecology* 25:897-922
- Glendinning JI (2007) How do predators cope with chemically defended foods? *Biological Bulletin* 213:252-266
- Goad DL, Pecquet ME, Warren HB (1989) Total serum-calcium concentrations in rabbits. *Journal of the American Veterinary Medical Association* 194:1520-1521
- Graça M, Bärlocher F (2005) Radial diffusion assay for tannins. In: Graça M, Bärlocher F, Gessner M (eds) *Methods to Study Litter Decomposition*. Springer Netherlands, pp 101-105
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous Inference in General Parametric Models. *Biometrical Journal* 50:346-363
- Hui CA (2004) Geophagy and potential contaminant exposure for terrestrial vertebrates. *Reviews of Environmental Contamination and Toxicology*, vol 183, pp 115-134
- Johns T (1990) *With bitter herbs they shall eat it : chemical ecology and the origins of human diet and medicine*. University of Arizona Press, Tucson
- Johns T, Duquette M (1991) Detoxification and mineral supplementation as functions of geophagy. *American Journal of Clinical Nutrition* 53:448-456
- Klaus G, Klaus-Hugi C, Schmid B (1998) Geophagy by large mammals at natural licks in the rain forest of the Dzanga National Park, Central African Republic. *Journal of Tropical Ecology* 14:829-839
- Klute A (ed) (1986) *Methods of soil analysis Part 1: Physical and mineralogical methods*, 2 edn. American Society of Agronomy and Soil Science. Society of America, Madison, WI
- Krebs CJ, Boonstra R, Boutin S, Sinclair ARE (2001) What drives the 10-year cycle of snowshoe hares? *Bioscience* 51:25-35
- Kreulen DA (1985) Lick use by large herbivores: A review of benefits and banes of soil consumption. *Mammal Review* 15:107-123
- Krishnamani R, Mahaney WC (2000) Geophagy among primates: adaptive significance and ecological consequences. *Animal Behaviour* 59:899-915
- Kubota J, Rieger S, Lazar VA (1970) Mineral Composition of Herbage Browsed by Moose in Alaska. *The Journal of Wildlife Management* 34:565-569
- McArthur C, Hagerman AE, Robbins CT (1991) Physiological strategies of mammalian herbivores against plant defenses. In: Palo R, Thomas, Robbins C, T. (eds) *Plant defenses against mammalian herbivory*. CRC Press, Inc., Boca Raton, pp 103-114
- National Research Council (1977) *Nutrient requirements of rabbits: 2nd rev. edn*. The National Academies Press

- National Research Council (2005) Mineral tolerance of animals: 2nd rev. edn. National Academies Press
- O'Donoghue M, Boutin S, Krebs CJ, Zuleta G, Murray DL, Hofer EJ (1998) Functional responses of coyotes and lynx to the snowshoe hare cycle. *Ecology* 79:1193-1208
- Page AL (ed) (1982) Methods of soil analysis Part 2: Chemical and microbiological properties. American Society of Agronomy and Soil Science. Society of America, Madison, WI
- Pehrson A (1983) Digestibility and retention of food components in caged mountain hares *Lepus timidus* during the winter. *Holarctic Ecology* 6:395-403
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2012) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-105
- Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge
- R Core Team (2012) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria
- Robbins CT (1993) Wildlife feeding and nutrition, 2nd edn. Academic Press, San Diego
- Robertson PG, Coleman DC, Bledsoe CS, Sollins P (eds) (1999) Standard soil methods for long-term ecological research. Oxford University Press, New York
- Rohner C (1995) Great horned owls and snowshoe hares - What causes the time-lag in the numerical response of predators to cyclic prey. *Oikos* 74:61-68
- Shan G (2011) Immunoassay applications in soil monitoring. John Wiley & Sons, Inc., Hoboken, NJ
- Slamova R, Trckova M, Vondruskova H, Zrally Z, Pavlik I (2011) Clay minerals in animal nutrition. *Applied Clay Science* 51:395-398
- Stenseth NC, Falck W, Bjornstad ON, Krebs CJ (1997) Population regulation in snowshoe hare and Canadian lynx: Asymmetric food web configurations between hare and lynx. *Proceedings of the National Academy of Sciences of the United States of America* 94:5147-5152
- Tracy BF, McNaughton SJ (1995) Elemental analysis of mineral lick soils from the Serengeti National Park, the Konza Prairie and Yellowstone National Park. *Ecography* 18:91-94
- Underwood EJ, Suttle NF (2001) The mineral nutrition of livestock, 3rd edn. CABI Pub., Wallingford, Oxon, UK ; New York, NY, USA
- USDA (1954) Diagnosis and improvement of saline and alkali soils. In: USDA (ed), Washington, DC, p 105
- Van Soest PJ (1994) Nutritional ecology of the ruminant, 2nd edn. Comstock Pub., Ithaca
- Wilson MJ (2003) Clay mineralogical and related characteristics of geophagic materials. *Journal of Chemical Ecology* 29:1525-1547
- Wolff JO (1978) Food habits of snowshoe hares in interior Alaska. *The Journal of Wildlife Management* 42:148-153
- Young SL, Sherman PW, Lucks JB, Pelto GH (2011) Why On Earth?: Evaluating Hypotheses About The Physiological Functions Of Human Geophagy. *The Quarterly Review of Biology* 86:97-120

- Young V, Hume ID (2005) Nitrogen requirements and urea recycling in an omnivorous marsupial, the borthern brown bandicoot *Isoodon macrourus*. *Physiological & Biochemical Zoology* 78:456-467
- Ziadi N, Tran TS (2008) Mehlich 3 extractable elements. In: Carter MR, Gregorich EG (eds) *Soil sampling and methods of analysis*, 2nd edn. CRC Press, Boca Raton, FL



### Chapter 3 Effects of geophagy or calcium supplementation on forage intake and body mass of snowshoe hares (*Lepus americanus*) in winter

#### Abstract

Geophagy is hypothesized to have several benefits, including detoxification of plant secondary metabolites and acquisition of mineral nutrients. Both sodium (Na) and calcium (Ca) are frequently cited as drivers of geophagy. A previous study showed that Na is an important component of a known lick near Wiseman, Alaska. Here, I study whether Ca is an important component of this lick. Daily forage intake of winter dormant *Salix alaxensis* twigs varied among hares offered  $\text{CaCO}_3$ ,  $\text{CaCl}_2$  or mineral soil ( $82.0 \pm 4.3$ ,  $52.3 \pm 5.2$ , and  $67.9 \pm 6.0 \text{ g kg}^{-0.75}$ , respectively). Hares offered  $\text{CaCl}_2$  lost more weight ( $6.3 \pm 1.0 \%$ ) than those offered  $\text{CaCO}_3$  ( $2.1 \pm 0.8 \%$ ). Hares lost  $3.1 \pm 1.5 \%$  when offered soil, but this was not significantly different than  $\text{CaCO}_3$  or  $\text{CaCl}_2$  treatments. Ca intake was highest for hares offered  $\text{CaCO}_3$ . Across treatments, Ca intake had a significant positive relationship with both forage intake and body mass. Increasing  $\text{CaCO}_3$  intake, from  $\text{CaCO}_3$  supplement or soil resulted in better weight maintenance but did not increase forage intake. Herbivores engaging in geophagy at this lick may realize the benefits of  $\text{CaCO}_3$ , as well those of Na.

## Introduction

Local observations in the Wiseman, Alaska area (N 67.41, W 150.11) suggest that snowshoe hares (*Lepus americanus*) consume mineral soil at specific sites.

Observers report that snowshoe hare populations in areas with a known lick appear to reach higher densities during the population high compared to areas where there is no known lick, indicating that access to licks confers a benefit. Geophagy is hypothesized to have several physiological benefits, including detoxification of plant secondary metabolites (PSMs) and acquisition of mineral nutrients (Krishnamani and Mahaney 2000; Slamova et al. 2011; Young et al. 2011).

Sodium (Na) is one of the primary minerals reported to trigger geophagy (e.g. Ayotte et al. 2006; Young et al. 2011; Dudley et al. 2012). Sodium is essential for many physiological processes, and because it does not accumulate in high concentrations in most plants, herbivores may be driven to seek out concentrated sources of Na such as licks (Hui 2004). Results from a previous study, described in chapter 2, indicate that Na is the major driver of soil intake during summer when hares feed on willow leaves (*Salix alaxensis*) and other deciduous species. However, soil is a complex matrix and herbivores may benefit from other elemental components of the lick as well.

Many licks, including the one under consideration here, often contain high concentrations of calcium (Ca). This element is frequently cited as a motivating factor in geophagic behaviors (e.g. Jones and Hanson 1985; Holl and Bleich 1987; Wilson 2003; Abrahams 2005; Ayotte et al. 2006; Young et al. 2011). Calcium is involved in muscle contraction, nerve impulse transmission and metabolism (Barboza et al. 2009). It is also

an essential element in the skeletal matrix, milk, antlers and eggshells, and is in high demand during times of growth and production.

Tannins, a class of plant secondary metabolites common in willows and other browse species used by hares, have been shown to reduce apparent absorption of Ca (Mitjavila et al. 1977; Freeland et al. 1985; Chang et al. 1994; Al-Mamary et al. 2001; Hassan et al. 2003), suggesting that animals consuming high tannin diets may benefit from a readily available Ca source, such as a lick. However, Ca interacts with phosphorus (P), reducing the availability of dietary P at high Ca:P ratios (Barboza et al. 2009). Considering this, excess Ca obtained through geophagy may actually be detrimental to herbivores. As shown in chapter 2, high Ca:P ratios correspond to reduced P retention and increasing weight loss in geophagic hares.

The aim of this study was to examine the role of Ca in the beneficial nature of geophagy. Specifically, I examined whether provision of Ca supplements is therapeutic, relative to soil from a known lick, for snowshoe hares fed a natural browse diet in winter (*S. alaxensis* twigs). Two forms of Ca were chosen for this study: (1) calcium carbonate ( $\text{CaCO}_3$ ) because it is often cited as a motivating factor in geophagy and is a common addition to livestock feed, both as a mineral supplement and to improve dry matter and starch digestibility in ruminants (Varner and Woods 1972; Cullison 1975; James and Wohlt 1985) and intake and weight gain in pigs (Patience and Wolynetz 1990); (2) calcium chloride ( $\text{CaCl}_2$ ) because there is abundant anecdotal evidence that hares congregate and eat soil at the edges of roads treated with  $\text{CaCl}_2$ . I hypothesized that soil



would be the superior supplement, but that  $\text{CaCO}_3$  would also confer a benefit to hares consuming winter browse.

## Methods

Ten snowshoe hares were captured and housed under UAF Animal Care and Use Committee protocol # 175963-6, as described in chapter 2. They were housed in 1 m x 2 m pens, on wood shavings. Hares were randomly assigned to 3 treatments groups. Treatments consisted of a single feeding regime of 100% winter dormant willow twigs and one of three mineral treatments: lick soil,  $\text{CaCl}_2$ , or  $\text{CaCO}_3$ . Hares were transitioned from the maintenance diet (see chapter 2) to the twig diet over six days. During the subsequent six-day experimental period, each group received a different mineral treatment. The experiment was repeated twice so that each group received each mineral treatment once. There were 14 days between each experimental period during which time hares were fed the maintenance diet for eight days before beginning the six-day transition to the twigs (Fig 3.1).

Small diameter ( $\leq 4$  mm), juvenile form willow twigs were collected and stored frozen in sealed plastic bags for use in the feeding trial. All twigs were used within 7 days of collection. Twigs were left intact (~15-45 cm) and offered ad libitum. Twigs were sampled daily for N, total phenolic and tannin analyses. Mineral supplements (soil and Ca) were offered in bricks prepared by adding gelatin (Knox brand, Kraft Foods, Tarrytown, NY) to the supplement at a rate of 1% by weight.

Refused forage and soil were collected daily to calculate intake. Hares were

weighed at the beginning, middle, and end of each treatment to calculate change in body mass (FD3H, Ohaus Corporation, Parsippany, NJ). Ambient air temperatures were recorded hourly (Thermochron iButton Device, Maxim Integrated, San Jose, CA).

### *Chemical analysis and calculations*

Willow samples were freeze-dried and ground in a Wiley Mill (#20 screen). Total phenolic concentration was determined by extraction in 70% acetone and using Folin-Ciocalteu reagent, as described by Ainsworth and Gillespie (2007). Tannin concentration was assessed by measuring the protein binding capacity, using the radial diffusion method (Graça and Bärlocher 2005).

All statistical analyses were executed using R (version 2.15.2; R Core Team 2012). Repeated measures using mixed effects models (Pinheiro et al. 2012) were used to compare daily forage and mineral intake and body mass change among mineral treatments. Pairwise contrasts were corrected for multiple comparisons with a Bonferroni adjustment (Hothorn et al. 2008). Square root transformations were applied to mineral intakes to correct for unequal variance related to the mean (Quinn and Keough 2002). Average values are reported as mean  $\pm$  SE. Statistical significance was determined at  $\alpha < 0.05$ .

## **Results**

Mean daily temperature during experimental periods ranged from -4.2°C to -33.8°C, and varied significantly among all experimental periods ( $P < 0.001$ ; Fig 3.2).

Averaged across trials, willow twigs contained  $10.5 \pm .14 \text{ mg g}^{-1} \text{ N}$ , had a total phenolic concentration of  $87.03 \pm 0.97 \text{ mg g}^{-1}$  gallic acid equivalents and a tannin concentration of  $47.62 \pm 1.34 \text{ mg g}^{-1}$  tannic acid equivalents. Nitrogen ( $P=0.1$ ), phenolic ( $P=0.5$ ) and tannin ( $P=0.2$ ) concentrations did not vary significantly among trials.

Hares with access to soil ingested  $14.7 \pm 1.5 \text{ g kg}^{-0.75}$  soil daily (Fig 3.3). Soil ingestion was significantly higher than ingestion of either Ca supplement ( $P<0.001$ ). Hares ate more  $\text{CaCO}_3$  ( $1.0 \pm 0.2 \text{ g kg}^{-0.75}$ ) than  $\text{CaCl}_2$  ( $0.2 \pm 0.1 \text{ g kg}^{-0.75}$ ) but the difference was not significant ( $P=0.52$ ; Fig 3.3). Hares offered  $\text{CaCO}_3$  ingested more Ca from supplements than hares offered soil or  $\text{CaCl}_2$  ( $P<0.001$ ; Fig 3.4), but they ingested more  $\text{CaCO}_3$  in soil than they did when offered pure  $\text{CaCO}_3$  ( $P<0.001$ ; Fig 3.5). Cl intake in hares on the  $\text{CaCl}_2$  treatment was  $0.15 \pm 0.05 \text{ g kg}^{-0.75}$ .

Daily forage intake varied among all mineral treatments ( $P\leq 0.01$ ; Fig 3.6). It was highest in hares with access to  $\text{CaCO}_3$  ( $82.0 \pm 4.3 \text{ g kg}^{-0.75}$ ) and lowest in hares with access to  $\text{CaCl}_2$  ( $52.3 \pm 5.2 \text{ g kg}^{-0.75}$ ). Forage intake for hares with access to soil was intermediate ( $67.9 \pm 6.0 \text{ g kg}^{-0.75}$ ). Phenolic concentration ( $P=0.001$ ) and temperature ( $P=0.004$ ) were significant covariates of forage intake, with forage intake increasing as total phenolic concentration and mean temperature decreased. Across treatments, forage intake was highest ( $78.4 \pm 6.0 \text{ g kg}^{-0.75}$ ) during the second experimental period, which was the coldest period. There was no difference in forage intake between the first and third experimental periods ( $57.3 \pm 7.4 \text{ g kg}^{-0.75}$  and  $61.7 \pm 7.4 \text{ g kg}^{-0.75}$ , respectively;  $P=0.8$ ), although temperature varied significantly (Fig 3.2).

Mean pre-trial body mass was  $1407 \pm 26$  g. On average, hares lost weight during each trial, regardless of mineral treatment. Hares lost  $6.3 \pm 1.0$  % of their body mass when offered  $\text{CaCl}_2$  compared to  $2.1 \pm 0.8$  % when offered  $\text{CaCO}_3$  ( $P \leq 0.01$ ; Fig 3.7). Hares lost  $3.1 \pm 1.5$  % when offered soil, but this was not significantly different from either the  $\text{CaCO}_3$  ( $P=1$ ) or  $\text{CaCl}_2$  ( $P=0.07$ ) treatments. High food intake rates during the recovery periods immediately following the experimental periods (Table 3.1) resulted in rapid recovery of body mass. Without exception, individuals gained more weight during the recovery period than they lost during the experimental period and were heavier at the start of each subsequent experimental period.

Supplement constituents had varying relationships with forage intake and weight maintenance. Across treatments, the relationship between  $\text{CaCO}_3$  intake and forage intake was not significant (Fig 3.8c), but increasing  $\text{CaCO}_3$  intake resulted in better weight maintenance ( $P=0.008$ ; Fig 3.8d). Calcium intake had a significant positive relationship with both forage intake ( $P=0.001$ ; Fig 3.8a) and body mass ( $P=0.008$ ; Fig 3.8b), although these relationships appear to be driven by the  $\text{CaCO}_3$  treatment.

## Discussion

Soil is a complex matrix that likely contains multiple beneficial constituents, in addition to constituents or properties that may be counterproductive or even harmful (Hui 2004; Abrahams 2005). Whereas the results of chapter 2 showed that Na was the primary beneficial constituent of soil from the mineral lick near Wiseman, Alaska, results from

the present experiment indicate that the beneficial nature of geophagy may not be limited to Na.

CaCl<sub>2</sub> was selected for study in this experiment in part because hares are reported to congregate along roadsides where CaCl<sub>2</sub> is used for dust control. Presumably hares derive some benefit from ingestion of treated road material. However, in pigs, CaCl<sub>2</sub> has been shown to reduce weight gain due to Cl induced acidosis (Yen et al. 1981). The reported intake reduction in those experiments is consistent with my results. However their experimental diets contained high CaCl<sub>2</sub> concentration (4%) and I saw no significant relationship between Cl intake and forage intake or weight. In my experiment, CaCl<sub>2</sub> contributed  $2.39 \pm 1.01$  g Cl kg<sup>-1</sup> DM, which is well below 20-50 g Cl kg<sup>-1</sup> DM, the dietary threshold considered excessive for poultry and pigs (Suttle 2010). Chlorine toxicity is thus an unlikely explanation of my observation that hares using CaCl<sub>2</sub> lost weight.

Across treatments, Ca ingestion corresponded with higher forage intake rates and decreased weight loss (Fig 3.8a, Fig 3.8b). However, these relationships appear to be driven largely by the CaCO<sub>3</sub> treatment, suggesting an effect of CaCO<sub>3</sub> rather than Ca per se. Although Ca is often cited as a motivating factor in geophagic behaviors, this typically occurs in animals with an elevated demand, such as those that lay eggs (Hui 2004). Wintering snowshoe hares are not expected to have high Ca demand. Furthermore, excess Ca can bind phosphate and reduce the dietary P availability, causing P imbalances (Barboza et al. 2009). Reduced P availability is a particular concern in rabbits and other hindgut fermenters, where intestinal Ca absorption is not regulated and

high dietary calcium results in increased blood Ca (National Research Council 2005).

The results of chapter 2 are consistent with P loss as a result of excess Ca intake. Hares were in negative P balance when fed willow, which contained twice as much Ca as the formulated diet. When fed the formulated ration, provision of soil resulted in >50% reduction of P digestibility. Across diets, increasing Ca:P ratios corresponded to increasing loss of body mass. I suspect that similar relationships exist in the present trial, given that P concentrations in willow twigs are lower than in leaves (Kubota et al. 1970) but Ca ingestion was similarly high.

Despite the apparently negative effect of Ca in geophagy, carbonates in the form of  $\text{CaCO}_3$  may be a beneficial constituent. Hares offered  $\text{CaCO}_3$  ingested several times more Ca than those on other treatments (Fig 3.4), but had the highest forage intake (Fig 3.6). Although  $\text{CaCO}_3$  intake was highest for hares offered soil (Fig 3.5), they ate less forage than hares offered pure  $\text{CaCO}_3$  (Fig 3.6), probably a result of increased gut fill for hares offered soil. Despite lower forage intake rates, hares on the soil treatment did not lose significantly more body mass than hares offered  $\text{CaCO}_3$  (Fig 3.7), suggesting that soil constituents other than  $\text{CaCO}_3$  contribute to the benefit. This conclusion is consistent with chapter 2 results where Na was found to be important. However,  $\text{CaCO}_3$  does seem to provide a benefit, as demonstrated by the positive relationship between weight gain and  $\text{CaCO}_3$  intake across treatments (Fig 3.8d). Although hares with the highest  $\text{CaCO}_3$  intake were those offered soil, the range and magnitude of weight loss was similar between the  $\text{CaCO}_3$  and soil treatments, and the positive relationship between body mass

change and  $\text{CaCO}_3$  intake remained when only hares offered  $\text{CaCO}_3$  were considered ( $y=6.54x-8.46$ ;  $P=0.005$ ).

The lack of a significant relationship between  $\text{CaCO}_3$  intake and forage intake (Fig 3.8c) suggests that  $\text{CaCO}_3$  improves forage assimilation.  $\text{CaCO}_3$  has been shown to improve cellulose, starch and crude protein digestion in ruminants fed a high concentrate diet, (Varner and Woods 1972; James and Wohlt 1985) and carbonates found in licks are believed to help wild ungulates minimize acidosis during spring forage change when diets contain high proportions of fermentable carbohydrates (Ayotte et al. 2006). However, these effects are generally attributed to altered microbial function and rumen digestion. In pigs, diets containing increasing concentrations of  $\text{CaCO}_3$  or  $\text{NaHCO}_3$  (sodium bicarbonate) have resulted higher daily gains associated with an increase in alkalinogenicity of the diet (Patience and Wolynetz 1990). These results appear to be mediated at least in part by changes in appetite, rather than improvement in forage assimilation. Across treatments, my regression data do not indicate that increasing  $\text{CaCO}_3$  in the diet results in higher food intake (Fig 3.8c) but this may be due to the effect of gut fill for hares offered soil. Although I did not quantify absorption of phenolic compounds or other PSMs, the effect of alkaline agents in the diets of browsing herbivores may be an important consideration, given the capacity for PSMs in these diets to create acid loads and increased the demand for bicarbonate used in neutralization and excretion of PSM metabolites (Foley et al. 1995).

$\text{CaCO}_3$  has been shown to increase apparent Na and K absorption in sheep (Yano et al. 1979). It is expected that hares will be Na deficient when fed a natural diet and

would benefit from improved absorption.  $\text{CaCO}_3$  has also been shown to improve apparent P absorption (James and Wohlt 1985), which would be beneficial in resolving Ca:P imbalance described in chapter 2. Improvements in Na and P acquisition and retention are consistent with my observation of better weight maintenance in hares offered  $\text{CaCO}_3$ .

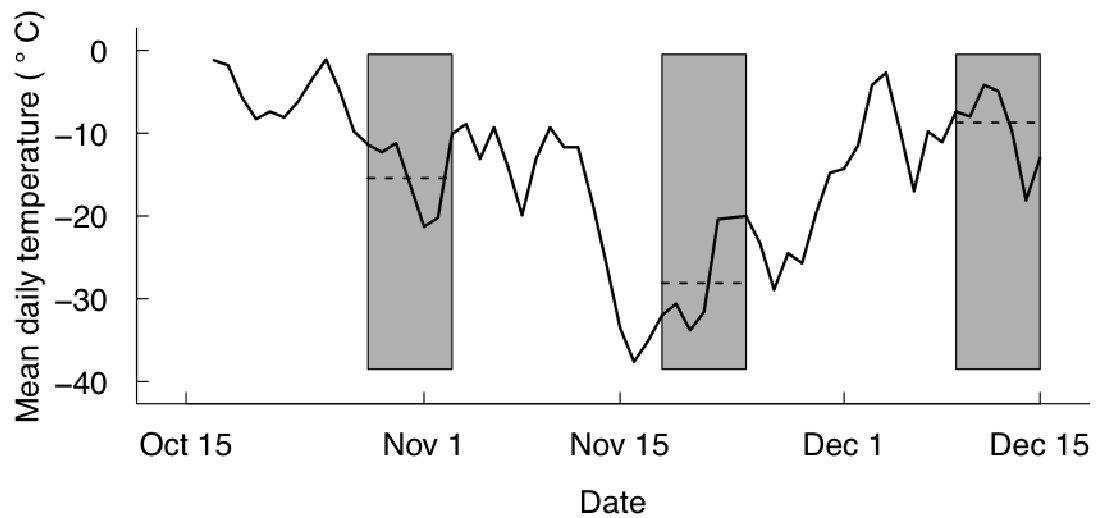
This experiment demonstrates that the benefits of soil cannot necessarily be attributed to a single soil component. Specifically it shows that, in addition to the Na benefit demonstrated in chapter 2,  $\text{CaCO}_3$  likely contributes to the therapeutic nature of soil ingestion at this lick, possibly via its effects on appetite and weight gain, or through interactions with other minerals. To further elucidate the role of carbonates as a therapeutic constituent of licks, I propose a series of trials measuring body mass, food intake, nutrient and energy assimilation and acid-base status of hares fed a browse diet, supplemented with one of several carbonates, bicarbonates (e.g.  $\text{CaCO}_3$ ,  $\text{NaHCO}_3$ ) or other mineral salts (e.g.  $\text{NaCl}$ ). Instead of providing ad libitum access to mineral supplements, daily intake could be regulated to provide either an equivalent amount of buffer or an equivalent amount of a given element. This would allow clarification of the benefits associated with mineral acquisition versus those associated with maintaining proper acid-base relations.



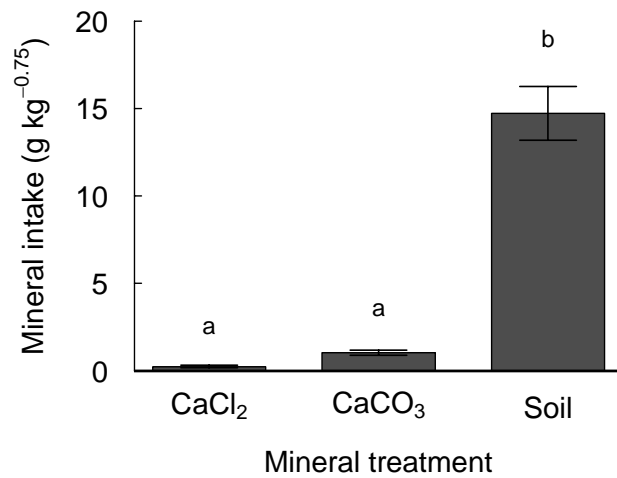
**Figures**

<b>Duration (days)</b>	<b>6</b>	<b>6</b>	<b>8</b>	<b>6</b>	<b>6</b>	<b>8</b>	<b>6</b>	<b>6</b>
<b>Group 1 n=4</b>	Transition to Willow	Soil	Maintenance Diet	Transition to Willow	CaCl <sub>2</sub>	Maintenance Diet	Transition to Willow	CaCO <sub>3</sub>
<b>Group 2 n=3</b>		CaCl <sub>2</sub>			CaCO <sub>3</sub>			Soil
<b>Group 3 n=3</b>		CaCO <sub>3</sub>			Soil			CaCl <sub>2</sub>

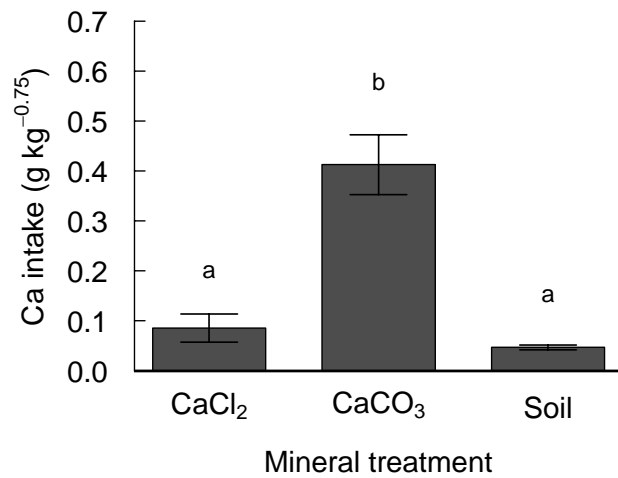
**Fig 3.1** Experimental design. Shaded areas indicate experimental periods.



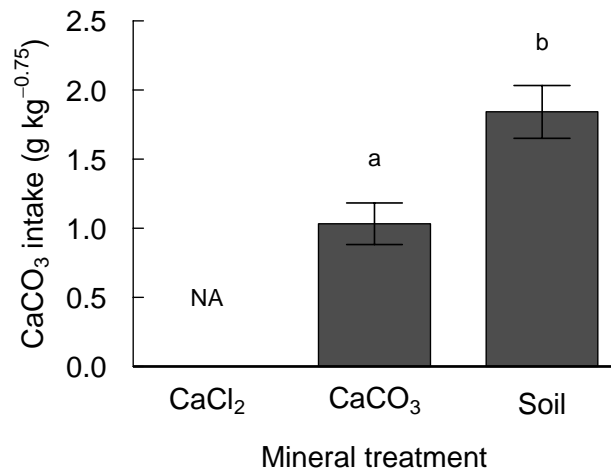
**Fig 3.2** Mean daily temperature (°C) at the hare facility during winter 2011. Shaded bars indicate experimental periods and dashed line indicates mean temperature for each period.



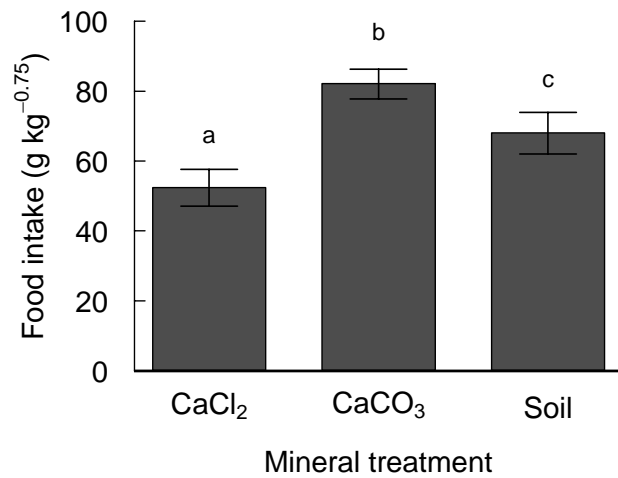
**Fig 3.3** Daily intake (g dry mass kg<sup>-0.75</sup>) of mineral supplement or soil by snowshoe hares fed winter dormant willow twigs (mean  $\pm$  SE; n=10). Different letters indicate significant difference among treatments ( $P < 0.001$ ).



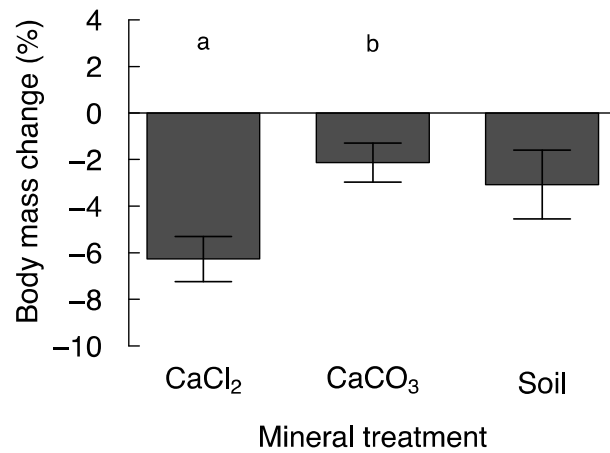
**Fig 3.4** Daily calcium intake (g Ca kg<sup>-0.75</sup>) from mineral supplements in snowshoe hares fed winter dormant willow twigs (mean  $\pm$  SE; n=10). Different letters indicate significant difference among treatments (P<0.001).



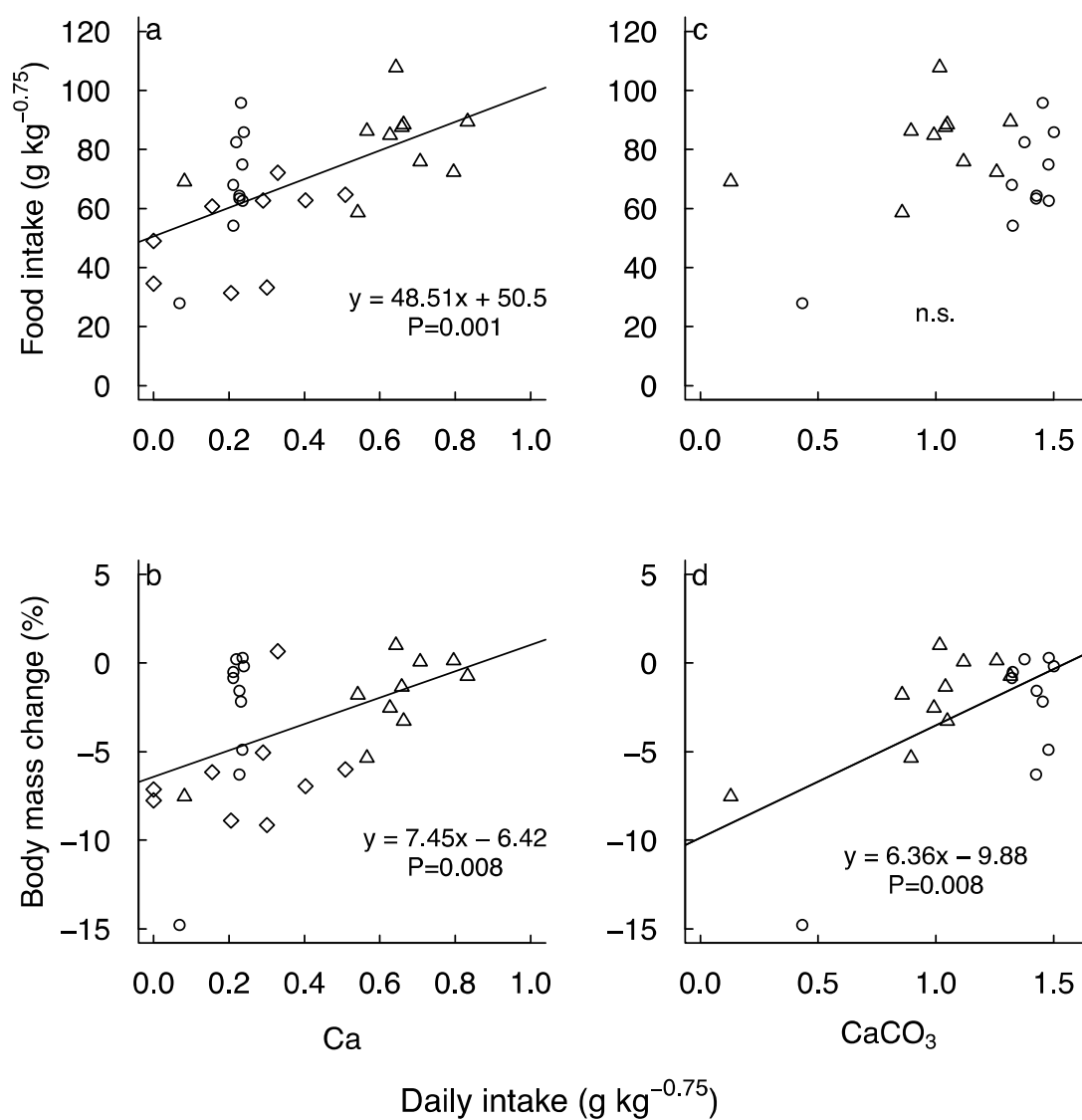
**Fig 3.5** Daily carbonate intake (g CaCO<sub>3</sub> kg<sup>-0.75</sup>) from mineral supplements in snowshoe hares fed winter dormant willow twigs (mean  $\pm$  SE; n=10). Different letters indicate significant difference among treatments (P<0.001).



**Fig 3.6** Daily forage intake (g dry mass kg<sup>-0.75</sup>) by snowshoe hares fed winter dormant willow twigs (mean ± SE; n=10). Different letters indicate significant difference among treatments ( $P \leq 0.01$ ).



**Fig 3.7** Change in body mass (%) of snowshoe hares fed winter dormant willow twigs and offered calcium or soil supplements (mean  $\pm$  SE;  $n=10$ ). Different letters indicate significant difference among treatments ( $P \leq 0.01$ ).



**Fig 3.8** Regressions of forage intake and body mass change on daily intake of supplement constituents for hares offered soil (circles), CaCO<sub>3</sub> (triangles), or CaCl<sub>2</sub> (squares).



**Tables**

	Pre-trial	1 <sup>st</sup> recovery period	2 <sup>nd</sup> recovery period
Food intake (g kg <sup>-0.75</sup> )	48.6 ± 1.9 <sup>a</sup>	81.7 ± 5.5 <sup>b</sup>	78.2 ± 6.2 <sup>b</sup>
Temperature (°C)	-0.7 ± 0.7 <sup>a</sup>	-12.2 ± 1.2 <sup>b</sup>	-20.3 ± 2.0 <sup>c</sup>

**Table 3.1** Daily intake rates and mean temperatures during non-experimental periods when hares were fed the maintenance diet. Different letters denote significant differences among periods (P<0.001).

## References

- Abrahams PW (2005) Geophagy and the involuntary ingestion of soil. In: Selinus O, Alloway BJ (eds) *Essentials of medical geology*. Elsevier Science, Amsterdam
- Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protocols* 2:875-877
- Al-Mamary M, Al-Habori M, Al-Aghbari A, Al-Obeidi A (2001) In vivo effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption. *Nutrition Research* 21:1393-1401
- Ayotte JB, Parker KL, Arocena JM, Gillingham MP (2006) Chemical composition of lick soils: Functions of soil ingestion by four ungulate species. *Journal of Mammalogy* 87:878-888
- Barboza PS, Parker KL, Hume ID (2009) *Integrative wildlife nutrition*. Springer-Verlag, Berlin ; New York
- Chang MCJ, Bailey JW, Collins JL (1994) Dietary tannins from cowpeas and tea transiently alter apparent calcium absorption but not absorption and utilization of protein in rats. *Journal of Nutrition* 124:283-288
- Cullison A (1975) *Feed and Feeding*. Prentice-Hall, Reston, VA
- Dudley R, Kaspari M, Yanoviak SP (2012) Lust for Salt in the Western Amazon. *Biotropica* 44:6-9
- Foley WJ, McLean S, Cork SJ (1995) Consequences of biotransformation of plant secondary metabolites on acid-base metabolism in mammals - a final common pathway. *Journal of Chemical Ecology* 21:721-743
- Freeland WJ, Calcott PH, Geiss DP (1985) Allelochemicals, minerals and herbivore population size. *Biochemical Systematics and Ecology* 13:195-206
- Graça M, Bärlocher F (2005) Radial diffusion assay for tannins. In: Graça M, Bärlocher F, Gessner M (eds) *Methods to Study Litter Decomposition*. Springer Netherlands, pp 101-105
- Hassan IAG, Elzubeir EA, El Tinay AH (2003) Growth and apparent absorption of minerals in broiler chicks fed diets with low or high tannin contents. *Tropical Animal Health and Production* 35:189-196
- Holl SA, Bleich VC (1987) Mineral lick use by mountain sheep in the San Gabriel Mountains, California. *Journal of Wildlife Management* 51:383-385
- Hothorn T, Bretz F, Westfall P (2008) Simultaneous Inference in General Parametric Models. *Biometrical Journal* 50:346-363
- Hui CA (2004) Geophagy and potential contaminant exposure for terrestrial vertebrates. *Reviews of Environmental Contamination and Toxicology*, vol 183, pp 115-134
- James LG, Wohlt JE (1985) Effect of Supplementing Equivalent Cation Amounts from NaCl, MgO, NaHCO<sub>3</sub> and CaCO<sub>3</sub> on Nutrient Utilization and Acid-Base Status of Growing Dorset Lambs Fed High Concentrate Diets. *Journal of Animal Science* 60:307-315
- Jones RL, Hanson HC (1985) Mineral licks, geophagy, and biogeochemistry of North American ungulates. The Iowa State University Press, Ames, IA

- Krishnamani R, Mahaney WC (2000) Geophagy among primates: adaptive significance and ecological consequences. *Animal Behaviour* 59:899-915
- Kubota J, Rieger S, Lazar VA (1970) Mineral Composition of Herbage Browsed by Moose in Alaska. *The Journal of Wildlife Management* 34:565-569
- Mitjavila S, Lacombe C, Carrera G, Drerache R (1977) Tannic acid and oxidized tannic acid on the functional state of rat intestinal epithelium. *The Journal of Nutrition* 107:2113-2121
- National Research Council (2005) Mineral tolerance of animals: 2nd rev. edn. National Academies Press
- Patience JF, Wolynetz MS (1990) Influence of dietary undetermined anion on acid-base status and performance in pigs. *Journal of Nutrition* 120:579-587
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Development Core Team (2012) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-105
- Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge
- R Core Team (2012) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria
- Slamova R, Trckova M, Vondruskova H, Zraly Z, Pavlik I (2011) Clay minerals in animal nutrition. *Applied Clay Science* 51:395-398
- Suttle NF (2010) Mineral Nutrition of Livestock. CABI
- Varner LW, Woods W (1972) Effect of Calcium and Starch Additions upon Ration Digestibility by Steers. *Journal of Animal Science* 35:410-414
- Wilson MJ (2003) Clay mineralogical and related characteristics of geophagic materials. *Journal of Chemical Ecology* 29:1525-1547
- Yano H, Matsui H, Kawashima R (1979) Effects of dietary calcium levels on concentration and solubility of macro minerals in the digestive tract of sheep. *Journal of Animal Science* 48:954-960
- Yen JT, Pond WG, Prior RL (1981) Calcium chloride as a regulator of feed intake and weight gain in pigs. *Journal of Animal Science* 52:778-782
- Young SL, Sherman PW, Lucks JB, Pelto GH (2011) Why On Earth?: Evaluating Hypotheses About The Physiological Functions Of Human Geophagy. *The Quarterly Review of Biology* 86:97-120

## Chapter 4 Conclusions

As many researchers have previously pointed out, lick soils are complex matrices which may contain multiple beneficial constituents (Hui 2004; Abrahams 2005). Among these are clay, which has a high adsorption capacity and may bind tannins and reduce dietary or endogenous nitrogen loss (Bernays et al. 1989). These soils also contain various mineral components, which have the capacity to ameliorate deficiencies arising from insufficient intake or increased demand (Young et al. 2011). However, mineral soils are typically abrasive, causing tooth wear and scouring of the intestinal tract. Furthermore, excess minerals can create imbalances and induce deficiencies (Underwood and Suttle 2001; Hui 2004; Abrahams 2005; Young and Hume 2005). I have shown that geophagy appears to provide a net benefit to snowshoe hares fed a natural browse diet, though both benefits and detriments were observed.

My research suggests that the therapeutic nature of geophagy appears to be related primarily to mineral nutrition rather than the direct mediation of tannins. The main benefit is attributable to improved sodium (Na) nutrition. Although the concentration of available Na in lick soil was lower than the dietary concentration considered adequate for domestic rabbits, soil contained more available Na than willow. Provision of soil increased digestible Na intake and elevated the ratio of Na to potassium (K). These results are consistent with previous studies showing that low dietary Na or poor Na retention result in weight loss and impaired dry matter and protein digestibility (Pehrson 1983; Chamorro et al. 2007). Given the importance of Na to physiological processes, and

its interactive constraint with PSMs, Na is a likely driver of geophagy at this lick. A Na drive may also explain the high daily soil intake rates. Although estimates of soil ingestion in free ranging small mammals are scarce, they are well below my observations in captive hares (Beyer et al. 1994).

The role of elemental calcium (Ca) as a driver of geophagy is less clear. High concentrations of dietary Ca in geophagic hares resulted in an imbalance with phosphorus (P). Increasing Ca:P ratios corresponded to reduced P retention and increasing weight loss. However, increasing  $\text{CaCO}_3$  intake, either from soil or in pure form, resulted in decreased weight loss. This is consistent with findings that pigs fed  $\text{CaCO}_3$  eat more and gain weight faster.  $\text{CaCO}_3$  has also been shown to increase apparent Na, K and P absorption (Yano et al. 1979; James and Wohlt 1985). Improvements in Na nutrition and Ca:P ratios are consistent with my observation of better weight maintenance in hare offered  $\text{CaCO}_3$ . Although my experimental design does not allow me to make conclusions about the mechanism responsible for the observed benefits of  $\text{CaCO}_3$  ingestion, I suggest that it does play a role in the therapeutic nature of this lick.

Changes in snowshoe hare survival as a result of geophagy may have implications ecosystem-wide. Hares are considered a keystone species of the boreal forest and their abundance affects both vegetation communities and predator densities (Bryant et al. 1991; Rohner 1995; Stenseth et al. 1997; O'Donoghue et al. 1998; Krebs et al. 2001). Given that food resources play an important role in regulating snowshoe hare population cycles (Bryant 1981; Krebs et al. 2001), and the potential for geophagy to alter use of food resources, licks may represent an important resource on the landscape and may

serve to increase the carrying capacity of the area (Klaus et al. 1998). Whereas the lick I studied is prominent, it is likely that many smaller, undetected licks exist in this area. It is unknown how prevalent these licks are, how they affect animal movement and spatial use of the food resources, and whether the benefits shown in my study are likely to be localized or widespread. These uncertainties notwithstanding, the abundance and distribution of licks may be an important factor controlling the local abundance and distribution of snowshoe hares in parts of interior Alaska.

## References

- Abrahams PW (2005) Geophagy and the involuntary ingestion of soil. In: Selinus O, Alloway BJ (eds) *Essentials of medical geology*. Elsevier Science, Amsterdam
- Bernays EA, Driver GC, Bilgener M (1989) Herbivores and plant tannins. *Advances in Ecological Research* 19:263-302
- Beyer WN, Connor EE, Gerould S (1994) Estimates of soil ingestion by wildlife. *Journal of Wildlife Management* 58:375-382
- Bryant JP (1981) Phytochemical deterrence of snowshoe hare browsing by adventitious shoots of 4 Alaskan trees. *Science* 213:889-890
- Bryant JP, Provenza FD, Pastor J, Reichardt PB, Clausen TP, Dutoit JT (1991) Interactions between woody-plants and browsing mammals mediated by secondary metabolites. *Annual Review of Ecology and Systematics* 22:431-446
- Chamorro S, Gomez-Conde MS, Carabano R, de Blas JC (2007) Short communication. Low dietary sodium content affects the digestibility of nutrients and fattening performance in growing rabbits. *Spanish Journal of Agricultural Research* 5:470-473
- Hui CA (2004) Geophagy and potential contaminant exposure for terrestrial vertebrates. *Reviews of Environmental Contamination and Toxicology*, vol 183, pp 115-134
- James LG, Wohlt JE (1985) Effect of Supplementing Equivalent Cation Amounts from NaCl, MgO, NaHCO<sub>3</sub> and CaCO<sub>3</sub> on Nutrient Utilization and Acid-Base Status of Growing Dorset Lambs Fed High Concentrate Diets. *Journal of Animal Science* 60:307-315
- Klaus G, Klaus-Hugi C, Schmid B (1998) Geophagy by large mammals at natural licks in the rain forest of the Dzanga National Park, Central African Republic. *Journal of Tropical Ecology* 14:829-839
- Krebs CJ, Boonstra R, Boutin S, Sinclair ARE (2001) What drives the 10-year cycle of snowshoe hares? *Bioscience* 51:25-35
- O'Donoghue M, Boutin S, Krebs CJ, Zuleta G, Murray DL, Hofer EJ (1998) Functional responses of coyotes and lynx to the snowshoe hare cycle. *Ecology* 79:1193-1208
- Pehrson A (1983) Digestibility and retention of food components in caged mountain hares *Lepus timidus* during the winter. *Holarctic Ecology* 6:395-403
- Rohner C (1995) Great horned owls and snowshoe hares - What causes the time-lag in the numerical response of predators to cyclic prey. *Oikos* 74:61-68
- Stenseth NC, Falck W, Bjornstad ON, Krebs CJ (1997) Population regulation in snowshoe hare and Canadian lynx: Asymmetric food web configurations between hare and lynx. *Proceedings of the National Academy of Sciences of the United States of America* 94:5147-5152
- Underwood EJ, Suttle NF (2001) *The mineral nutrition of livestock*, 3rd edn. CABI Pub., Wallingford, Oxon, UK ; New York, NY, USA
- Yano H, Matsui H, Kawashima R (1979) Effects of dietary calcium levels on concentration and solubility of macro minerals in the digestive tract of sheep. *Journal of Animal Science* 48:954-960

- Young SL, Sherman PW, Lucks JB, Pelto GH (2011) Why On Earth?: Evaluating Hypotheses About The Physiological Functions Of Human Geophagy. *The Quarterly Review of Biology* 86:97-120
- Young V, Hume ID (2005) Nitrogen requirements and urea recycling in an omnivorous marsupial, the borthern brown bandicoot *Isodon macrourus*. *Physiological & Biochemical Zoology* 78:456-467